NITROTYROSINE AND E-SELECTIN STATUS WITH REFERENCE TO OTHER MARKERS OF ENDOTHELIAL DYSFUNCTION(VEGF-A, NITRIC OXIDE, ENDOTHELIN) IN TYPE 2 DIABETES MELLITUS

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LIST OF ABBREVIATIONS

AGEs	Advanced glycation end products
BMI	Body Mass Index
CKD	Chronic Kidney Disease
DKA	Diabetic Ketoacidosis
DM	Diabetes Mellitus
DN	Diabetic Nephropathy
EC	Endothelial cells
eGFR	Estimated Glomerular Filtration Rate
ELISA	Enzyme- Linked Immuno Sorbent Assay
eNOS	Endothelial Nitric Oxide Synthase
ESRD	End Stage Renal Disease
ET	Endothelin
FBS	Fasting Blood Sugar
GDM	Gestational Diabetes Mellitus
GLUT	Glucose Transporter
HbA1C	Glycated Hemoglobin
HDL	High Density Lipoprotein
IDF	International Diabetes Federation
IR	Insulin Resistance
KDIGO	Kidney Disease Improving Global Outcomes
LDL	Low Density Lipoprotein
MAPK	Mitogen- activated protein kinase

MDA	Malondialdehyde
NO	Nitric Oxide
NT	Nitrotyrosine, also known as 3-Nitrotyrosine
OGTT	Oral glucose tolerance test
PPBS	Post Prandial Blood Sugar
QUICKI	Quantitative Insulin-Sensitivity Check Index
RNS	Reactive nitrogen species
ROC curve	Receiver Operating Characteristic curve
ROS	Reactive Oxygen Species
TC	Total Cholesterol
TG	Triglycerides, also known as Triacylglycerols
T2 DM	Type 2 Diabetes Mellitus
UACR	Urine albumin-to-creatinine ratio
VEGF	Vascular Endothelial Growth Factor
VLDL	Very -Low- Density Lipoprotein

1. INTRODUCTION

Diabetes mellitus (DM) is a serious metabolic and endocrine disorder. Genetic predisposition, irregular eating habits, sedentary lifestyle and obesity are some of the causes, which in association with other factors including environmental considerations account for a pronounced morbidity and higher mortality(1).

Diabetes is characterized by a reduced tolerance for glucose. The classification, diagnostic criteria, methods of diagnosis and treatment modalities of diabetes have vastly improved over the last two decades, and newer inroads have been made in this direction.Equally significant are the well laid down programs for enhancing awareness, prevention, and control.According to IDF, 2006, globally 285 million people had diabetes, and by the year 2030, this number is expected to increase to 438 million. It is expected by 2025 that around 250 million would be affected with Diabetes, as per a report from the American Diabetes Association(2).

Citing the census of year 2010, there were around 344 million persons worldwide who had impaired glucose tolerance. By year 2030, this number could reach a staggering 472 million (3). Children under 19 years are afflicted by an increasing incidence of type 1 diabetes (T1DM). Presently, type 2 diabetes (T2DM) is posing a serious global health issue that looms largely over the world economy with reference to developed as well as developing nations.This trend could be attributed to the globally prevalent fast culture, behavioral changes, rapid urbanization, sedentary lifestyle, and most significantly unhealthy and unsafe practices(4).

DM was taken cognizance of, as early as in 1500 BCE. DM was known by the conventional name *madhumeh*. Diabetes in the ancient Indian medicine, namely Ayurveda literally denotes *sweet urine*. The Romans called it a diarrhea of pee, or frequent urination(5). Mellitus is synonymous with the term *sweet urine* and Diabetes has been linked to other illnesses also.Sushruta and Charaka, two ancient Indian doctors had segregated DM into two categories(6).

In the early 1920s, Frederick Banting and Charles Best madethe epoch-making discovery of insulin under the stewardship of John Macleod at the University of Toronto. With the assistance of James Collip, insulin was purified, thus rendering it available as a therapeutic modality for diabetes.Banting and Bestrealized a significant milestone in the annals of DM, with reference to the therapeutic modality for DM. History in the realms of DM treatment was further fortified when insulin was synthesized(7).

Pioneering studies by Sanger and co-workers during the productive period 1945–1955 culminated in the elucidation of the amino acid sequence and subsequently the comprehensive structure of insulin from many a species came to the fore.Insulin is one among the first peptides to be accorded the status of a hormone(8).Incidentally, insulinrepresents the first peptide whose structure was unraveledand this became the forerunner to the concept and development of structural analysis of proteins .

Hyperglycemia is synonymous with DM. For an unambiguous diagnosis of DM to be made, the fasting blood glucose must be 7.0mmol/l or above (126mg/dl or above).It is a universally accepted fact that the ideal parameter to measure plasma glucose that satisfies the international requirements pertaining to either the diagnosis or the monitoring of DM is fasting blood sugar [FBS] or fasting blood glucose [FBG] (9).

The sustained presence of hyperglycemia and the associated dyslipidemia inflict damage to the blood vessels and elevatethe risk of organ functional disorders. The complications include diabetic nephropathy (DN), neuropathy and retinopathy. It is documented that the DM associated microvascular aberration might be present if the patient has had diabetes for a duration more than five years(10). About 13% of all diabetics in India possess diabetic nephropathy. End-stage renal disease (ESRD), a life-threatening condition, manifests from nephropathy.In ESRD, the kidney is incapacitated and hence the elimination of endogenous and exogenous substances including toxins becomes a limiting event (11).

Conventionally, FBS, HbA1C, renal function tests and lipid profile have figured in the armamentarium of biochemical investigations(12).Presently, Urine albumin-to-creatinine ratio (UACR) and HbA1C measurements are considered essential for objectively assessing the extent of renal cell damage in diabetic nephropathy,as exemplified by increased excretion of urine albumin, altered UACR, oedema and lowered immunity(13).Hence, it is imperative to necessitate early biomarkers to detect renal damage at the appropriate time that would mitigate kidney damage and prolong life. It would largely prove to be beneficial to diabetics if we could detect even a semblance of renal cell inflammation or damage, much prior to the onset of pronounced albuminuria(14).

The complications of DM including hyperglycemia (glucotoxicity) and dyslipidemia (lipotoxicity) enhance the generation of free radicals induced by oxidative stress. A nexus featuring oxidative stress and diabetic complications is responsible for organ (renal) damage, as per the evidences accumulating from both experimental and clinical investigations(15).The aetiology of DN and the development of end-stage renal disease (ESRD) are both significantly influenced by oxidative stress.

Endothelial failure has been envisaged primarily as one that is linked to glomerular enlargement and mesangial expansion. Endothelin-1 and Vascular Endothelial Growth Factor (VEGF), which are both generated in abnormal quantities,are synonymous with angiogenesis and vaso permeability. Evidences are available to quote the fact that Endothelin-1 and circulating VEGF-A contribute to the glomerular capillary hyperpermeability of macromolecules.This in all probability would largely denote the etiology of diabetic albuminuria(16).Endothelin-1 (ET-1) and Vascular Endothelial Growth Factor-A (VEGF-A) have been recognized as markers of endothelial vascular dysregulated metrics,with insulin resistance (IR)forming the core. DN could be classified in a graded manner, based on albuminuria, oxidative stress and inflammation(17).

We undertook the present study to probe into the status of nitrotyrosine and E selectin along with the other markers of endothelial dysfunction in defining DN with respect to UACR and estimated Glomerular Filtration rate (eGFR), basically with the

intention of finding out a reliable as well as an objective modality for early detection of kidney disease in diabetics(18).

Several cardinal biochemical players are implicated in the genesis of reactive oxygen species in the vascular system.Of these, the role played by endothelial nitric oxide synthase (eNOS) is considered cardinal in the generation of reactive oxygen species (ROS) and Reactive Nitrogen species (RNS). This event is triggered by cytokines and stress(19). Based on the available evidences, it is in the fitness of things to state that Nitric oxide might play a huge role in endothelial dysfunction, culminating in diabetic nephropathy.

2. NEED FOR THE PRESENT STUDY AND SCOPE OF THE INVESTIGATION

Diabetic kidney disease (DKD) essentially comprises diabetic nephropathy (DN), non-diabetic renal diseases (NDRD), intermediate forms (DN & NDRD), and even scenario in conjunction with T2DM that include hypertension, vascular disease etc. Endothelial dysfunction has been widely implicated in cardiovascular and renal morbidity associated with type 2 DM. Hence, any study in this direction would have wide ramifications with impending implications. Very few studies from South India are presently available that link the markers of endothelial dysfunction to the morbidity and magnitude of diabetic nephropathy. The present study isan earnest attempt that has laid due emphasis on endothelial function/distress in diabetic nephropathy, based on the objective stratification of thegroups, with reference to albuminuria.

The diagnosis of Diabetic nephropathy (DN) has been conventionally based on the presence of micro-albuminuria (MA). However, the value of MA is hampered by the fact that in order to adequately predict Diabetic Kidney Disease particularly in youngsters or in non-albuminuric kidney disease, additional biomarkers of glomerular renal injury have to be enabled. This would help uncover early renal dysfunction, besides providing an insight into the morphological aberrations, even before MA becomes apparent. This calls for the need to define novel and reliable predictive biomarkers to augment urinary albumin excretion (UAE), especially during the preliminary stages of diabetic kidney disease which would then provide a gamut of opportunities for promulgating timely preventive and/or therapeutic interventions. This is imperative with a view to delaying the onset and progression of irreversible long-term complications. Hence, the present study of ours acquires even more relevance.

3-Nitrotyrosine (NT) has been recognized as a marker of oxidative stress/nitrosative stress in diabetes mellitus. NT has also been studied in variegated metabolic conditions. The aim of the study was essentially oriented towards the role of NT as a predictor of oxidative stress mediated impending nephropathy in diabetes mellitus and that with due reference to albuminuria.

E-selectin, a cell adhesion molecule depicting nexus with oxidative stress in hyperglycemia has been documented in several other clinical conditions. The present study has attempted to project the role of E-selectin as a biomarker of diabetic nephropathy,as linked to oxidative stress.

Endothelin-1 (ET-1) and Vascular Endothelial Growth Factor-A (VEGF-A) have been recognized as markers of endothelial dysfunction in diabetes mellitus. VEGF-A and ET-1 are vasoactive peptides that have a nexus with vascular manifestations. This study compared vascular dysregulated metrics,VEGF-A and ET-1 with IR and insulin sensitivity, in the light of risk factors for diabetic nephropathy, but in a graded manner. Albuminuria was employed as the yardstick for enabling the gradation/ phases of nephropathy. In this study, we made an attempt to decipher the relationship between various phases of diabetic nephropathy and the endothelial dysfunction markers, namely VEGF-A and Endothelin-1, in the light of albuminuria.

The nexus between urine protein and serum nitric oxide (with reference to the central role mediated by IR has been evaluated in type 2 diabetics by earlier groups of workers. However, in the present study, we wanted to elicit the value of nitric oxide as a solitary, reliable marker of oxidative stress/ nitrosative stress mediated endothelial dysfunction in nephropathy, besides its projected relationship with other biochemical parameters that are altered due to IR and glycemic control.

3. AIM AND OBJECTIVES

Aim

- To study the significance of Nitrotyrosine, E-Selectin with reference to VEGF-A, Nitric oxide, Endothelin as markers of endothelial dysfunction in type 2 diabetic patients with reference to nephropathy

Objectives

- To measure the plasma glucose level in fasting and post prandial condition(FBS, PPBS), Lipid profile, parameters related to renal function and glycemic control, IR in T2DM patients and compare with healthy individuals, in the light of Urine Albumin -to- creatinine ratio (UACR)
- To quantitate the extracellular levels of Nitrotyrosine, E-selectin,VEGF-A, Endothelinand Nitric oxide in T2DM patients and compare the samewith healthy individuals for possible association involving UACR and estimated Glomerular Filtration rate (eGFR)
- To study the probable association involving the above-mentioned parameters with reference to the conventional indices of insulin sensitivity/IR, in the light of eGFR and UACR
- To assess as to how IR would behave according to the levels of related categorical variables, in the light of eGFR and UACR, for the purpose of objectivizing the diagnostic and prognostic relevance of the markers of endothelial dysfunction
- To arrive at an outcome with reference to personalized medicine for managing diabetic nephropathy, based on biochemical considerations and magnitude of albuminuria

4. RESEARCH QUESTION

- What would be the significance of quantitating Nitrotyrosine, E-Selectin with reference to other markers of endothelial dysfunction, namely VEGF-A, Nitric oxide, and Endothelinin T2DM, with particular reference to nephropathy, based on the objective categorisation of the study subjects into three groups, namely normo, -micro- and macroalbuminuric?

5. REVIEW OF LITERATURE

Diabetes Mellitus:

Diabetes mellitus, a metabolic disease characterized by high blood sugar is a serious global health issue that is casting a financial burden on the society. Consumption of high-calorie diet, fast food and sedentary life style are causing an alarming rise in obesity, which in turn promotes IR in tissues such as the liver, muscle, and fat(20).

Asia is the epicenter of the global type-2 diabetes epidemic, with China and India ranking first and second, respectively. Diabetes mellitus affects about 463 million people aged 20 to 79 worldwide, according to the International Diabetes Federation (IDF), with the number predicted to grow to 578.4 million by 2030 and 700 million by 2045 (IDF, 2019). The number of people with type-2 diabetes is increasing in most countries, and 79 percent diabetics hail from low-and middle – income countries.

The countries with the highest numbers of diabetics (aged 20–79years) are China (116.4 million), India (77 million), and the United States of America (31 million) and are expected to remain so in 2030. It has been predicted that the number of diabetics in Pakistan(19.4 million at present) will increase to 36 million by 2045. Diabetes affects 77 million people in India, with the number expected to rise to 101 million by 2030 and 134.2 million by 2045 (IDF, 2019).

Diabetes mellitus develops as a result of factors that impede insulin synthesis, secretion and function (21).However, T2DM is synonymous with IR. The complications of DM, in general include ketoacidosis, hypoglycemia, hyper osmolarity, lactic acidosis, and long-term vascular complications, with the latter being the culmination of IR.

Table-1 - Etiologic classification of diabetes mellitus(As per American Diabetes Association)(22)

I. Type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency)	**II.Type 2 diabetes** (might range from pre-dominantly IR with relative insulin deficiency to a pre-dominantly secretory defect with IR)	**III.Other specific types** A.Genetic defects of β-cell function B.Genetic defects in insulin action C.Diseases of the exocrine pancreas D.Endocrinopathies E.Drug or chemical induced F.Infections G.Uncommon forms of immune-mediated diabetes H.Other genetic syndromes	V. **Gestational diabetes mellitus**

The long -term complications resulting from ineffective management of T2DM could be classified into microvascular complications, namely retinopathy, neuropathy and nephropathy, or macrovascular complications. The macrovascular aberrations include cardiovascular, cerebrovascular, and peripheral vascular diseases (Peripheral Arterial Occlusive Disease).

Diabetic Nephropathy:

Diabetic nephropathy (DN) is a serious and debilitating condition that culminates in chronic renal failure. It is life threatening. End-stage renal failure is more obvious in nephropathy associated with DM. According to the International Diabetes Federation (IDF), 40% of diabetics might end up having renal failure.Furthermore, about 80% of end-stage renal failure is attributed to diabetes and uncontrolled hypertension. Asians record higher rates of chronic renal disease. The relationship governing vascular disease, end-stage renal disease, and diabetes mellitus has been dealt with in detail (23).

Proteinuria is the key indicator of ESRD (Wiles et al. 2019). The excretion of albumin begins in the first few weeks following the onset of DM. The characteristic changes include mesangial cell expansion, fibrotic alterations in the interstitium, and an enhancement in the density of basement membrane. It is opined strongly, based on evidences, that urinary albuminuria and the estimated GFR (eGFR) could be regarded as appropriate parameters for facilitating both diagnosis and continuous monitoring (24).

Reactive oxygen species (ROS) are generated as a result of hyperglycemia, and the cardinal pathways such as protein kinase C, polyol and advanced glycation end products (AGEs) are activated to varying extents. A myriad of cytokines and chemokines are implicated in events that alter vascular permeability. Albuminuria is an off shoot of podocytopathy. Proteinuria results in the metamorphosis of epithelial cells into mesenchymal cells, synonymous with the production of connective tissue cells and progressive tubular damage. Persistent albuminuria on two or more occasions, separated by a minimum period of three months in early-morning urine samples is used to identify and confirm diabetic nephropathy. A sign of early DN is moderately elevated albuminuria, which occurs when the 24-hour urinary albumin excretion rate is between 30 and 300 mg. However, urinalysis is mandatory to rule out a urinary tract infection as the cause of albuminuria (25).

Nitrotyrosine (NT) is indirectly inferred as a marker of oxidative stress because of the production of peroxynitrite, also called as peroxonitrite ($ONOO^-$) during acute hyperglycemia. It is well documented that oxidative stress and the pathogenesis of IR through insulin signal inhibition and adipocytokines dysregulation are considered cardinal in this regard.

The evaluation of albuminuria and glomerular filtration rate (GFR) has become the mainstay, as recommended by the KDIGO: Kidney Disease Improving Global Outcomes CKD Work Group. The evaluation is with reference to end stage renal disease (ESRD) which is presently on the rise globally and hence it is imperative to monitor the progression of diabetic nephropathy.

Careful monitoring of albuminuria is advocated in routine practice in diabetes, and it is underlined that GFR declines in both type 1 and type 2 diabetes. In such a scenario, however, it is not pragmatic and safe to predict the natural course of diabetic nephropathy based on albuminuria and eGFR alone. It is with this perspective that the study was planned with a view to documenting the role of E-selectin as an objective biomarker, in combination with the conventional parameters, namely albuminuria and eGFR. E-selectin is considered as an indirect marker of oxidative stress that could be attributed to hyperglycemia. The inclusion of other markers of endothelial dysfunction in our study would further augment our efforts. This would fortify the prediction of vascular outcome as observed in diabetic nephropathy, thereby paving the way for upholding distinct relevance in the realms of evidence-based medicine.

E-selectin:

Selectins are adhesion molecules that play a pivotal role in cellular repair and also in inflammatory responses. The C-type mammalian lectin (glycoprotein) family includes selectins which bind to carbohydrates, a process dependent on the availability of the divalent cation, Calcium. Leukocyte (L)-selectin, platelet (P)-selectin, and endothelial (E)-selectin are the three members of the selectin subfamily. The amount of E-selectin in the extracellular fluid fluctuates based on blood pressure and glycemic control. Oxidative stress, which is believed to be the root cause of this, results in damage to the endothelium and the resultant release of circulating adhesion molecules like endothelin-1. This prompted the study of E-selectin in individuals with persistent T2DM(26). We wanted to extend the study to the monitoringof diabetic nephropathy.

Nitrotyrosine (NT):

Oxidative stress and free radical generation are rampant in DM and it has been documented that serum nitrotyrosine levels were perceptibly elevated in chronic DM with nephropathy. In a study on DN patients, it has been reported that while nitric oxide (NO) is ceaselessly generated and eliminated by the enzyme systems, increased production of $ONOO^{-}$results in the conversion of NO to NT, which in turn is central to DN (27).

Since serum nitrated protein has a half-life of only 1.6 hours, it cannot be quantified. However, the estimation of nitrotyrosine, a stable marker of oxidative stress is a viable alternative. NT levels are pronouncedly high in diabetics. Research has shown that the free radical peroxynitrite may harm renal cells and render the antioxidant enzyme systems inactive.

Oxidative stress/ nitrosative stress leads to the generation of higher quantities of nitrogenous molecules including protein. This in turn stimulates the levels of Nitric Oxide Synthase (NOS) enzyme and Nitric Oxide (NO). The existence of a pronounced shift in the eNOS protein expression in diabetic animal models has been noticed . Vascular injury is caused by an increase in nitrotyrosine deposition and this largely accounts for the progression of DN. In view of the above-mentioned facts and availability of limited studies in our chosen area, to the best of our knowledge, we had embarked on the study by referring to the literature available, as indicated in established databases (28).

Nitric oxide:

Nitric oxide (NO), a gaseous molecule secreted by the endothelium is a key player in regulating endothelial function. NO is synthesized from L-arginine by nitric oxide synthases (NOSs) viz. neuronal NOS (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS). NO possesses variegated actions. It is generally believed that low levels of NO enable physiological functions. On the contrary, high levels of NO might lead to deleterious effects. NO can react with superoxide anion to generate peroxynitrite radical, which binds to proteins and thus affects their function. DM is associated with enhanced levels of nitric oxide (29). NO is regarded as a putative biomarker for diabetics with the comorbidity frequently being hypertension. Nitric oxide levels increase as diabetic complications become much more pronounced. NO has also been implicated in cardiovascular diseases .Alterations in the levels of NO have been implicated in end-stage diabetic nephropathy.

Despite these considerations, very few reports are available in DM complications, with reference to the cross- talk involving NO and other markers of endothelial dysfunction. Hence, we undertook the study.

VEGF-A:

VEGF, earlier known as vascular permeability factor (VPF) is a homodimer glycoprotein that is highly conserved. VEGF enhances vascular permeability, besides involved in angiogenesis and mitogenesis. VEGF comprises VEGF-A, B, C, D, E and placental growth factor. Podocytes which are specialized epithelial cells are the major source of VEGF-A in kidney. VEGF-A expression is also perceived in mesangial cells and collecting ducts. Podocytes produce three VEGF-Aiso forms (VEGF 121, 165, and 189) in a constitutive manner. Studies have revealed that hyperglycemia associated with IR induces glomerular damage that is mediated by VEGF-A signalling and expression of NADPH oxidase 4 gene (30).

It must however be stated that endothelial dysfunction is not merely associated with atherosclerosis but also is involved in the manifestations related to renal vascular complications. Since DM is associated with endothelial dysfunction and also in view of the act that not many studies from South India are available in DM related nephropathy, we deemed it appropriate to study biochemically the various markers of endothelial function with the hope that we would be able to arrive at a logical conclusion, based on the stratification of albuminuria and eGFR.

Endothelin-1:

When generated in copious quantities, Endothelin-1 (ET-1) becomes synonymous with the regulation of angiogenesis and vaso permeability. Evidences are available to cite that ET-1 could contribute to the altered glomerular capillary hyper permeability with reference to macromolecules. This denotes diabetic albuminuria(31).

In the study that we had undertaken, based on the current understanding of association between endothelial dysfunction and progression of DN, we had evaluated the status of markers of endothelial (dys)function, viz., NO, Endothelin-1, VEGF-A and E- selectin, besides Nitro-tyrosine in T2DM with a view to assessing the marker molecules in the light of the biochemical stages of nephropathy and with reference to estimated glomerular filtration rate, glycemic control, IR and indices of insulin sensitivity.

6. SUBJECTS, STUDY DESIGN AND METHODOLOGY

The study was conducted at the work place of the Principal Investigator, namely the Department of Biochemistry, Konaseema Institute Of Medical Sciences and Research Foundation(KIMS & RF), Amalapuram, Andhra Pradesh, India,
The details pertaining to the finalization of the sample size are provided below.
SAMPLE SIZE CALCULATION (32)

$$n = \frac{Z^2 pq}{d^2}$$

Where n=Sample size
p=Prevalence=11.4%=0.114
q=1-p=1-0.114=0.886
d=0.045% at 95% confidence Interval
Z=1.96 at 5% level of significance
Then,

$$n = \frac{(1.96)^2 \text{ x}0.114\text{x}0.886}{(0.045)^2}$$

n =192

Sample size of present study (n)=200

A total of 670 patients were taken into due consideration. Among them,150 type 2 diabetics aged 35 to 55 yrs, who had visited the clinics at KIMS Amalapuram, EG Dist., AP, India fulfilled the criteria and comprised the Test group .All these subjects had a duration of DM that was five years or more and were maintained on standard medical management (oral hypoglycemic agents).

50 healthy individuals, age and gender matched constituted the Control group. Anthropometric parameters were measured and duly documented. Informed consent was obtained from both the groups, following a detailed explanation of the study in the vernacular language. A proper and comprehensive history was documented that

was fortified by a properly structured questionnaire, duly pre validated. Compliance with the Helsinki declaration of 1975 was enabled. The study was begun only following the approbation of the Research Advisory Committee at the institute where the principal investigator had registered himself as a doctoral candidate and also following clearance from the Institutional Human Ethics Committee**(IEC/PR/2015/142/015, dated: 14/12/2015)** at his workplace.

The following criteria were applied to the Test group (n=150)

Inclusion Criteria

- Diabetic patients(T2DM) aged between 35 to 55 years
- Patients on oral hypoglycemic drugs

Exclusion Criteria:

- Patients on all types of insulin therapy
- Known history of uncontrolled and resistant hypertension
- Smokers, alcoholics, tobacco chewers
- Subjects who had abnormal urinary sediment, urinary tract infection, history of other renal diseases
- Active or chronic persistent infection or inflammatory disorders, neoplastic disorders
- Thyroid disorders,
- Liver dysfunction,
- History of acute myocardial infarction, stroke, and occlusive peripheral vascular disease.

Study Design

For the purpose of estimation of urinary microalbumin and creatinine, the first (morning) urine samples were collected in sterile container and appropriately used for analysis. In addition to the Control Group, the test group subjects were segregated according to urine albumin-to- creatinine ratio (UACR) criteria(expressed in mg/g of creatinine). The details are depicted in Table 2.

Table 2: Stratification of study groups

Group	Description	Number	Criteria
Group – I	Controls	n= 50	
Group – II	DM with normoalbuminuria	n= 50	<30 mg/g creatinine
Group – III	DM with microalbuminuria	n=50	30–299 mg/g creatinine
Group – IV	DM with macroalbuminuria	n=50	≥300 mg/g creatinine

Biochemical analysis: 8 ml of venous blood samples were withdrawn from the antecubital vein from all the subjects, following an overnight fast, under aseptic conditions. Routine laboratory investigations were performed immediately and other aliquots were stored at −80 °C until further analyses of specific parameters, namely Nitrotyrosine,E-selectin, Nitric oxide,VEGF-A,Endothelin, and Insulin. Post prandial blood samples were collected appropriately for blood glucose analysis.

Methodology:

The summary of anthropometric, physiological methods employed in the present study isprovidedin Table -3

Table 3: Anthropometric andphysiological measurements

Parameter	Mode of assessment	Methods
Body Mass Index(BMI)	Computation	Kg/m2(33)
Waist circumference	Measuring tape	Aroundthenarrowestpartof the stomach (middle above the hip bone(34)
Waist hip ratio	Measuring tape	Waist in cms/Hip in cms(35)
Systolic Blood Pressure	Sphygmomanometer	(36)
Diastolic Blood Pressure	Sphygmomanometer	(36)

Reference values:

Body Mass Index(BMI) – 18.5 to 24.9kg/m^2

Waist circumference – 0.9 or less in men&0.85 or less for women

Systolic Blood Pressure -120 to 129 mm Hg

Diastolic Blood Pressure -80 to 84 mm Hg

Biochemical methods:

Plasma Glucose Estimation:

Plasma glucose was estimated by GOD-POD method utilizing ERBA Mannheim diagnostic kit on EM -200 (Transasia) fully automated analyzer (37).

The results are expressed in mg/dland in mmol/l, as deemed appropriate.

Estimation of urea in blood:

Urea(mg/dl)was estimated by Urease - GLDH method, end point by utilizing the ERBA Mannheim, diagnostic kit on EM -200 (Transasia) fully automated analyzer, as per the instructions of the manufacturer(38).

Estimation of Creatinine in serum:

Creatinine (mg/dl) in serum was estimated by Jaffe's kinetic methodusing ERBA Mannheim diagnostic kiton EM-200 (Transasia) fully automated analyzer (39).

Estimation of Total Cholesterol in serum :

Serum cholesterol(mg/dl) was estimated by CHOD-PAP method utilizing ERBA Mannheim diagnostic kit on EM-200 fully automated analyzer(40).

Estimation of Triglycerides in serum:

Serum triglycerides(mg/dl)wasestimated by GPO-method (end point)utilizing ERBA Mannheim diagnostic kiton EM-200 fully automated analyzer, as per the instructions provided by the manufacturer of the kit (41).

Estimation of HDL - Cholesterol in serum:

HDL cholesterol(mg/dl) was estimated by a method based on modified polyvinyl sulfonic acid (PVS) and polyethyleneglycol-methyl ether (PEGME) coupled classic precipitation byemployingERBA Mannheim diagnostic kit, on EM-200 fully automated analyzer.(42).

Quantitation of LDL – cholesterol:

This parameter was quantitatedutilizing Friedewaldformula(43).

Friedewald formula:

LDL cholesterol(mg/dl) = Total cholesterol – (HDL cholesterol + VLDL cholesterol)

Triglycerides/5 = VLDL cholesterol

Table 4: Reference values for the biochemical parameters

Biochemical Parameters	Reference Range
Plasma Glucose -Fasting(mg/ dl)	70to 100mg/dl
Plasma Glucose –Post Prandial(mg/ dl)	100 to 140 mg/dl
Plasma Insulin (mU/ml)	2-12 µU/mL
HbA1c (in %)	Lessthan 6% Normal (Above 6%Diabetes)
Total Cholesterol(mg/dl)	150 to 200mg/ dl
Triacylglycerols (mg/dl)	50 - 150mg/dl
High Density Lipoprotein(mg/dl)	30-80 mg/ dl
Low Density Lipoprotein(mg/dl)	Lessthan 130mg/dl Desirable Less than 100 mg/dl
Urea(mg/dl)	15-40 mg/dl
Creatinine(mg/dl)	0.7-1.3 mg/dl (Men) 0.7 -1.2 mg/dl (Women)
HOMA-IR(a surrogate of IR)	Above 1.9 indicates early IR Above 2.69 indicates significant IR
HOMA BETA	• Greater than 80:Normal • 55 - 80:Insulinresistance likely • Lessthan 55 (Diabetes diagnosis likely)
Quantitative Insulin Sensitivity check index (QUICKI)	• Greater than 0.45:Normal • 0.30-0.45:Insulinresistance likely • Lessthan 0.339:Associated with a trend towards increasing IR, obesity, and cardiovascular disease. • Lessthan0.30: (Diabetes diagnosis likely)
Triglyceride – Glucose index	Below 4
eGFR	The average eGFR in subjects in their 20s is about 116 mL/min/1.73m2. It drops to 85 mL/min/1.73m2 (in their 60s)
Urine albumin -to- creatinine ratio	Normoalbuminuria - <30 mg/g creatinine Microalbuminuria - 30–299 mg/g creatinine Macroalbuminuria - ≥300 mg/g creatinine
Nitrotyrosine	0 to 0.2 µmol/L
E-selectin	18 -35ng/ml
VEGF-A	27 to 90pg/ml
Endothelin	0.7 to 5pg/ml
Nitric oxide	10 to40µmol/L

Estimation of Hemoglobin A1C (Glycated Hemoglobin)

Whole blood was collected in EDTA tubes. HbA1c was estimated by enhanced immunoturbidimetric method by using ERBA Mannheim diagnostic kit on EM-200 autoanalyzer(44). The results expressed as %.

Estimation of Urine Microalbumin (spot urine)

For the estimation of microalbumin, the first (early morning voided) urine was collected in sterile container. Urinary microalbumin (mg/dl)was measured by turbidimetric immunoassay method utilizing ERBA Mannheim diagnostic kits on EM-200 (Transasia) fully automated analyzer (45).

Estimation of Urinary Creatinine (spot urine)

For the estimation of urine creatinine (mg/dl), freshly voided early morning sample of urine was collected in a sterile container. Creatinine in the urine was measured by Jaffe's kinetic method utilizing ERBA Mannheim, diagnostic kits on EM-200 (Transasia) fully automated analyzer.(46).

Urine Albumin –to- creatinine ratio(UACR)

Based on the assays of urine microalbumin (mg/dl) and urine creatinine (mg/dl), UACR was calculated and expressed in the standard units (mg/g of creatinine) (47).

Estimation of plasma insulin (Fasting):

Plasma insulin (µIu/ml) was quantitated based on an ELISA method and the kits were procured from M/s DiaMetra, Spello,PerugioItaly.LISA SCAN (Transasia) ELISA reader was utilised appropriately. Strict protocol was followed, as per the instructions of the manufacturer (48).

Estimation of Nitrotyrosine in serum :

Serum nitrotyrosine was estimated by ELISA method and thekits wereprocured from M/s Sincere Biotech Ltd, Beijing, China and utilizing LISA SCAN (Transasia) ELISA reader. The protocol followed was in compliance with the instructions provided by the manufacturer. The results were expressed as µmol/L(49)

Estimation of E-selectin in serum:

E-selectin in serum samples was quantitated using an ELISA method. The kits were procured fromBosterBio Ltd, USAandLISA SCAN (Transasia) ELISA reader was employed. The protocol was essentially as per the manufacturer instructions. The results were duly expressed in ng/ml(50).

Estimation of VEGF-A and Endothelin in serum:

VEGF-A and Endothelin were estimated in serum based on ELISA methods. The kits were procured fromM/s Sincere Biotech Co. Ltd., China and Boster Bio Ltd, USA respectively. The estimations were enabled using LISA SCAN (Transasia) ELISA reader. The results were expressed as pg/ml for both VEGF-A &Endothelinrespectively(51,52).

Estimation of Nitric oxide in serum:

The estimation was based on spectrophotometry (53) and the results were expressed in µmol/L

Homeostatic Model Assessment for Insulin Resistance (HOMA-IR):

HOMA -IR is a surrogatemarker for IR and was computed on the basis of plasma insulin and plasma glucose(venous) respectively. The formula put forth by Matthews et al. was essentially followed (54).

HOMA – IR = Fasting insulin (µU/ml) X fasting glucose (mg/dl)/405

HOMA –BETA

HOMA-beta, a measure of beta cell mass was computed on the basis of plasma glucose and insulin respectively. Venous plasma was used for the purpose and the samples were obtained essentially in the post-absorptive or fasting state

(HOMA-beta=(360 × fasting insulin (µU/mL))/(fasting glucose (mg/dl)-63).Glucose in mg/dl, Insulin (Fasting) in µU/ml(55).

The Triglyceride Glucose Index (TyG index) was determined using the formula [fasting triglycerides (mg/dL) × fasting plasma glucose (mg (dL)/2].

Quantitative Insulin-Sensitivity Check Index(QUICKI)

It was computed, as per the formula put forth by Katz et al(56)

QUICKI = 1/[log(Fasting Insulin in μU/ml) + log(Fasting Glucose in mg/dl)]

eGFR

It was calculated in compliance with CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) andusing the formula(57).

Table 5: Computation of estimated Glomerular Filtration Rate (eGFR)

	Serum Creatinine (mg/dl)	eGFR
Females	≤0.7	$144 \times (Scr/0.7)^{-329} \times (0.993)^{age}$
	>0.7	$144 \times (Scr/0.7)^{-1.209} \times (0.993)^{age}$
Males	≤0.9	$141 \times (Scr/0.9)^{-0.411} \times (0.993)^{age}$
	>0.9	$141 \times (Scr/0.9)^{-1.209} \times (0.993)^{age}$

7. STATISTICAL ANALYSIS

Statistical analysis was carried out by SPSS software, version-22 licensed to KIMS & RF (Data for continuous variables was expressed as mean ± SD; p value < 0.05 was considered statistically significant)

- Pearson correlation
- Receiver Operating Characteristic
- Two way ANOVA

8. RESULTS

The results pertaining to the mean and standard deviation of the various groups in terms of the biochemical parameters and the comparison of all the groups for determining statistical significance of the various parameters are duly indicated.

The results of the specific parameters such as Nitrotyrosine, E-selectin,VEGF-A, Endothelin and other biochemical parameters possessing different magnitudes of statistical significance when examined with control vs normoalbuminuric, control vs microalbuminuria, control vs macroalbuminuria, normoalbuminuric vs microalbuminuria, normoalbuminuric vs macroalbuminuria, microalbuminuria vs macroalbuminuria are indicated in Tables 6to 12.

Table-6: Groups Mean, Standard Deviations and associated 'p' values - Control (Group-I) vs Normoalbuminuria(Group-II)

Sl.no	Parameter	Control Group – I		Normoalbuminuria Group - II		'p' Value I vs II
		Mean	SD	Mean	SD	
1	Microalbumin (mg/dl)	16.94	1.58	19.74	2.83	0.0138
2	Urinary Creatinine (mg/dl)	88.86	4.06	85.38	9.43	0.0074
3	UACR (mg/g of creatinine)	19.1	1.94	23.27	3.4	0.0026
4	FBS(mg/dl)	83.96	5.36	135.12	16.29	0.0072
5	PPBS (mg/dl)	112.3	11.12	179.78	19.08	0.0080
6	HbA1c%	5.76	0.55	7.75	0.84	0.0014
7	Insulin(µIu/ml)	6.66	0.58	10.67	2.65	0.0038
8	HOMA-IR	1.38	0.14	3.59	1.07	0.0011
9	HOMA BETA	122.62	35.26	55.69	18.8	0.0021
10	QUICKI	0.36	0.01	0.32	0.01	0.0056
11	eGFR	116.01	16.68	108.98	29.69	0.0052
12	Urea (mg/dl)	25.12	3.94	27.78	4.07	0.0361
13	Creatinine(mg/dl)	0.73	0.17	0.78	0.24	0.0151
14	Cholesterol (mg/dl)	169	9.23	189.08	18.3	0.0024
15	Triglycerides(mg/dl)	98.3	7.22	119.1	28.64	0.0066
16	HDL-cholesterol (mg/dl)	42.72	2.65	39.38	3	0.0541
17	LDL-cholesterol (mg/dl)	106.62	10.22	125.88	14.82	0.0237
18	Nitrotyrosine(µmol/L)	0.21	0.03	0.28	0.05	0.0072
19	E-selectin(ng/ml)	23.12	3.81	35.38	6.46	0.0057
20	VEGF-A(pg/ml)	75	7.21	119.5	16.66	0.0050
21	Endothelin(pg/ml)	1.39	0.08	2.7	0.56	0.0026
22	Nitric oxide(µmol/L)	33.36	3.24	42.64	6.14	0.0024

UACR-Urine Albumin -Creatinine ratio; FBS-Fasting blood sugar(glucose);PPBS-Post prandial blood sugar(glucose); HbA1c- Glycated Hemoglobin; HOMA-IR- Homeostatic model assessment for Insulin resistance; HOMA-β- Homeostatic model assessment – Beta; QUICKI-Quantitative Insulin-Sensitivity Check Index; eGFR- Estimated Glomerular Filtration Rate;HDL-C—High Density Lipoprotein; LDL-Low Density Lipoprotein;VEGF-A-Vascular Endothelial Growth Factor A

P<0.05 – Significant and P<0.001 – highly significant**

Table-7: Groups Mean, Standard Deviations and associated 'p' values - Control (Group-I) vs Microalbuminuria (Group-III)

S.no	Parameter	Control Group – I		Microalbuminuria Group - III		'p' Value
		Mean	SD	Mean	SD	I vs III
1	Microalbumin (mg/dl)	16.94	1.58	77.72	11.66	**0.0002****
2	Urinary Creatinine (mg/dl)	88.86	4.06	63.52	19.28	**0.0007****
3	UACR (mg/g of creatinine)	19.1	1.94	132.51	38.57	**0.0003****
4	FBS(mg/dl)	83.96	5.36	149.5	27.98	0.0011
5	PPBS (mg/dl)	112.3	11.12	209.2	38.33	0.0024
6	HbA1c%	5.76	0.55	8.45	0.99	**0.0001****
7	Insulin(µIu/ml)	6.66	0.58	15.3	3.18	0.0012
8	HOMA-IR	1.38	0.14	5.69	1.68	**0.0002****
9	HOMA BETA	122.62	35.26	46.35	17.88	0.0011
10	QUICKI	0.36	0.01	0.3	0.02	0.0021
11	eGFR	116.01	16.68	85.9	24.11	0.0016
12	Urea (mg/dl)	25.12	3.94	31.66	4.21	0.0042
13	Creatinine(mg/dl)	0.73	0.17	0.97	0.31	0.0053
14	Cholesterol (mg/dl)	169	9.23	197.66	23.47	0.0089
15	Triglycerides(mg/dl)	98.3	7.22	136.86	28.63	0.0043
16	HDL-cholesterol (mg/dl)	42.72	2.65	38.62	2.13	0.0055
17	LDL-cholesterol (mg/dl)	106.62	10.22	131.67	22.88	0.0037
18	Nitrotyrosine(µmol/L)	0.21	0.03	0.36	0.1	**0.0007****
19	E-selectin(ng/ml)	23.12	3.81	67.74	8.69	**0.0006****
20	VEGF-A(pg/ml)	75	7.21	139.13	14.45	0.0010
21	Endothelin(pg/ml)	1.39	0.08	3.51	0.3	**0.0005****
22	Nitric oxide(µmol/L)	33.36	3.24	45.9	7.03	0.0031

UACR-Urine Albumin -Creatinine ratio; FBS-Fasting blood sugar(glucose);PPBS-Post prandial blood sugar(glucose); HbA1c- Glycated Hemoglobin; HOMA-IR- Homeostatic model assessment for Insulin resistance; HOMA-β- Homeostatic model assessment – Beta; QUICKI-Quantitative Insulin-Sensitivity Check Index; eGFR- Estimated Glomerular Filtration Rate; HDL-C—High Density Lipoprotein; LDL-Low Density Lipoprotein;VEGF-A-Vascular Endothelial Growth Factor A

P<0.05 – Significant and P<0.001 – highly significant**

Table-8: Groups Mean, Standard Deviations and associated 'p' values - Control (Group-I) vs Macroalbuminuria (Group-IV)

S.no	Parameter	Control Group – I		Macroalbuminuria Group - IV		'p' Value
		Mean	SD	Mean	SD	I vs IV
1	Microalbumin (mg/dl)	16.94	1.58	187.18	20.85	**0.0001****
2	Urinary Creatinine (mg/dl)	88.86	4.06	44.14	6.86	**0.0002****
3	UACR (mg/g of creatinine)	19.1	1.94	430.08	55.4	**0.0001****
4	FBS(mg/dl)	83.96	5.36	252.4	33	**0.0008****
5	PPBS (mg/dl)	112.3	11.12	331.8	30.1	**0.0009****
6	HbA1c%	5.76	0.55	9.53	0.49	**0.0002****
7	Insulin(µIu/ml)	6.66	0.58	18	2.27	**0.0006****
8	HOMA-IR	1.38	0.14	11.25	2.13	**0.0004****
9	HOMA BETA	122.62	35.26	35.39	6.75	**0.0008****
10	QUICKI	0.36	0.01	0.27	0.01	**0.0007****
11	eGFR	116.01	16.68	79.38	21.79	**0.0008****
12	Urea (mg/dl)	25.12	3.94	31.48	2.63	0.0012
13	Creatinine(mg/dl)	0.73	0.17	1.08	0.29	0.0045
14	Cholesterol (mg/dl)	169	9.23	209.64	21.73	0.0011
15	Triglycerides(mg/dl)	98.3	7.22	162.66	16.7	0.0024
16	HDL-cholesterol(mg/dl)	42.72	2.65	36.36	3.0	0.0044
17	LDL-cholesterol (mg/dl)	106.62	10.22	140.75	21.74	0.0036
18	Nitrotyrosine(µmol/L)	0.21	0.03	0.5	0.1	0.0010
19	E-selectin(ng/ml)	23.12	3.81	91.76	7.31	**0.0002****
20	VEGF-A(pg/ml)	75	7.21	173.38	10.32	**0.0006****
21	Endothelin(pg/ml)	1.39	0.08	5.11	0.49	0.0010
22	Nitric oxide(µmol/L)	33.36	3.24	58.56	10.82	0.0011

UACR-Urine Albumin -Creatinine ratio; FBS-Fasting blood sugar(glucose);PPBS-Post prandial blood sugar(glucose); HbA1c- Glycated Hemoglobin; HOMA-IR- Homeostatic model assessment for Insulin resistance; HOMA-β- Homeostatic model assessment – Beta; QUICKI-Quantitative Insulin-Sensitivity Check Index; eGFR- Estimated Glomerular Filtration Rate; HDL-C—High Density Lipoprotein; LDL-Low Density Lipoprotein;VEGF-A-Vascular Endothelial Growth Factor A

P<0.05 – Significant and P<0.001 – highly significant**

Table-9: Groups Mean, Standard Deviations and 'p' values - Normoalbuminuria (Group-II) vs Microalbuminuria (Group-III)

S.no	Parameter	Normoalbuminuria Group – II		Microalbuminuria Group - III		'p' Value
		Mean	SD	Mean	SD	II vs III
1	Microalbumin (mg/dl)	19.74	2.83	77.72	11.66	0.0010
2	Urinary Creatinine (mg/dl)	85.38	9.43	63.52	19.28	0.0024
3	UACR (mg/g of creatinine)	23.27	3.4	132.51	38.57	0.0018
4	FBS(mg/dl)	135.12	16.29	149.5	27.98	0.0067
5	PPBS (mg/dl)	179.78	19.08	209.2	38.33	0.0078
6	HbA1c%	7.75	0.84	8.45	0.99	**0.0001****
7	Insulin(µIu/ml)	10.67	2.65	15.3	3.18	**0.0002****
8	HOMA-IR	3.59	1.07	5.69	1.68	**0.0001****
9	HOMA BETA	55.69	18.8	46.35	17.88	**0.0010****
10	QUICKI	0.32	0.01	0.3	0.02	0.0021
11	eGFR	108.98	29.69	85.9	24.11	0.0010
12	Urea (mg/dl)	27.78	4.07	31.66	4.21	0.0167
13	Creatinine(mg/dl)	0.78	0.24	0.97	0.31	0.0210
14	Cholesterol (mg/dl)	189.08	18.3	197.66	23.47	0.0010
15	Triglycerides(mg/dl)	119.1	28.64	136.86	28.63	0.0011
16	HDL-cholesterol (mg/dl)	39.38	3	38.62	2.13	0.0270
17	LDL-cholesterol (mg/dl)	125.88	14.82	131.67	22.88	0.0301
18	Nitrotyrosine(µmol/L)	0.28	0.05	0.36	0.1	0.0010
19	E-selectin(ng/ml)	35.38	6.46	67.74	8.69	**0.0001****
20	VEGF-A(pg/ml)	119.5	16.66	139.13	14.45	**0.0002****
21	Endothelin(pg/ml)	2.7	0.56	3.51	0.3	**0.0006****
22	Nitric oxide(µmol/L)	42.64	6.14	45.9	7.03	**0.0001****

UACR-Urine Albumin -Creatinine ratio; FBS-Fasting blood sugar(glucose);PPBS-Post prandial blood sugar(glucose); HbA1c- Glycated Hemoglobin; HOMA-IR- Homeostatic model assessment for Insulin resistance; HOMA-β- Homeostatic model assessment – Beta; QUICKI-Quantitative Insulin-Sensitivity Check Index; eGFR- Estimated Glomerular Filtration Rate; HDL-C—High Density Lipoprotein; LDL-Low Density Lipoprotein;VEGF-A-Vascular Endothelial Growth Factor A

P<0.05 – Significant and P<0.001 – highly significant**

Table 10: Groups Mean, Standard Deviations and 'p' values - Normoalbuminuria (Group-II) vs Macroalbuminuria (Group-IV)

S.no	Parameter	Normoalbuminuria Group – II		Macroalbuminuria Group - IV		'p' Value
		Mean	SD	Mean	SD	II vs IV
1	Microalbumin (mg/dl)	19.74	2.83	187.18	20.85	**0.0001****
2	Urinary Creatinine (mg/dl)	85.38	9.43	44.14	6.86	**0.0003****
3	UACR (mg/g of creatinine)	23.27	3.4	430.08	55.4	0.0034
4	FBS(mg/dl)	135.12	16.29	252.4	33	0.0046
5	PPBS (mg/dl)	179.78	19.08	331.8	30.1	**0.0001****
6	HbA1c%	7.75	0.84	9.53	0.49	**0.0004****
7	Insulin(µIu/ml)	10.67	2.65	18	2.27	**0.0002****
8	HOMA-IR	3.59	1.07	11.25	2.13	**0.0007****
9	HOMA BETA	55.69	18.8	35.39	6.75	0.0056
10	QUICKI	0.32	0.01	0.27	0.01	0.0024
11	eGFR	108.98	29.69	79.38	21.79	0.0011
12	Urea (mg/dl)	27.78	4.07	31.48	2.63	0.0134
13	Creatinine(mg/dl)	0.78	0.24	1.08	0.29	0.0204
14	Cholesterol (mg/dl)	189.08	18.3	209.64	21.73	0.0024
15	Triglycerides(mg/dl)	119.1	28.64	162.66	16.7	0.0031
16	HDL-cholesterol (mg/dl)	39.38	3	36.36	3	0.0102
17	LDL-cholesterol (mg/dl)	125.88	14.82	140.75	21.74	0.0112
18	Nitrotyrosine(µmol/L)	0.28	0.05	0.5	0.1	0.0011
19	E-selectin(ng/ml)	35.38	6.46	91.76	7.31	**0.0002****
20	VEGF-A(pg/ml)	119.5	16.66	173.38	10.32	**0.0001****
21	Endothelin(pg/ml)	2.7	0.56	5.11	0.49	0.0025
22	Nitric oxide(µmol/L)	42.64	6.14	58.56	10.82	0.0010

UACR-Urine Albumin -Creatinine ratio; FBS-Fasting blood sugar(glucose);PPBS-Post prandial blood sugar(glucose); HbA1c- Glycated Hemoglobin; HOMA-IR- Homeostatic model assessment for Insulin resistance; HOMA-β- Homeostatic model assessment – Beta; QUICKI-Quantitative Insulin-Sensitivity Check Index; eGFR- Estimated Glomerular Filtration Rate; HDL-C—High Density Lipoprotein; LDL-Low Density Lipoprotein;VEGF-A-Vascular Endothelial Growth Factor A

P<0.05 – Significant and P<0.001 – highly significant**

Table-11: Groups Mean, Standard Deviations and 'p' values - Microalbuminuria (Group-III) vs Macroalbuminuria (Group-IV)

S.no	Parameter	Microalbuminuria Group – III		Macroalbuminuria Group - IV		'p' Value III vs IV
		Mean	SD	Mean	SD	
1	Micro Albumin (mg/dl)	77.72	11.66	187.18	20.85	0.0012
2	Urinary Creatinine (mg/dl)	63.52	19.28	44.14	6.86	0.0011
3	UACR (mg/g of creatinine)	132.51	38.57	430.08	55.4	**0.0001****
4	FBS(mg/dl)	149.5	27.98	252.4	33	0.0040
5	PPBS (mg/dl)	209.2	38.33	331.8	30.1	0.0033
6	HbA1c%	8.45	0.99	9.53	0.49	**0.0006****
7	Insulin(µIu/ml)	15.3	3.18	18	2.27	**0.0001****
8	HOMA-IR	5.69	1.68	11.25	2.13	**0.0004****
9	HOMA BETA	46.35	17.88	35.39	6.75	0.0011
10	QUICKI	0.3	0.02	0.27	0.01	0.0034
11	eGFR	85.9	24.11	79.38	21.79	0.0013
12	Urea (mg/dl)	31.66	4.21	31.48	2.63	0.0201
13	Creatinine(mg/dl)	0.97	0.31	1.08	0.29	0.0112
14	Cholesterol (mg/dl)	197.66	23.47	209.64	21.73	0.0163
15	Triglycerides(mg/dl)	136.86	28.63	162.66	16.7	0.0110
16	HDL-cholesterol (mg/dl)	38.62	2.13	36.36	3	0.0010
17	LDL-cholesterol (mg/dl)	131.67	22.88	140.75	21.74	0.0012
18	Nitrotyrosine(µmol/L)	0.36	0.1	0.5	0.1	0.0011
19	E-selectin(ng/ml)	67.74	8.69	91.76	7.31	**0.0001****
20	VEGF-A(pg/ml)	139.13	14.45	173.38	10.32	0.0013
21	Endothelin(pg/ml)	3.51	0.3	5.11	0.49	0.0016
22	Nitric oxide(µmol/L)	45.9	7.03	58.56	10.82	0.0011

UACR-Urine Albumin -Creatinine ratio; FBS-Fasting blood sugar(glucose);PPBS-Post prandial blood sugar(glucose); HbA1c- Glycated Hemoglobin; HOMA-IR- Homeostatic model assessment for Insulin resistance; HOMA-β- Homeostatic model assessment – Beta; QUICKI-Quantitative Insulin-Sensitivity Check Index; eGFR- Estimated Glomerular Filtration Rate; HDL-C—High Density Lipoprotein; LDL-Low Density Lipoprotein;VEGF-A-Vascular Endothelial Growth Factor A

P<0.05 – Significant and P<0.001 – highly significant**

Table-12: Comparison of all groups by ANOVA (one-way) for determining statistical significance of Biochemical parameters

S.no	Parameter	All Groups for ANOVA
1	Micro Albumin (mg/dl)	0.0011
2	Urinary Creatinine (mg/dl)	0.0012
3	UACR (mg/gm of creatinine)	0.0010
4	FBS(mg/dl)	0.0013
5	PPBS (mg/dl)	0.0017
6	HbA1c%	**0.0001****
7	Insulin(µIu/ml)	**0.0002****
8	HOMA-IR	**0.0001****
9	HOMA BETA	**0.0004****
10	QUICKI	**0.0001****
11	eGFR	**0.0010****
12	Urea (mg/dl)	0.0061
13	Creatinine(mg/dl)	0.0013
14	Cholesterol (mg/dl)	0.0015
15	Triglycerides(mg/dl)	0.0016
16	HDL-cholesterol (mg/dl)	0.0019
17	LDL-cholesterol (mg/dl)	0.0047
18	Nitrotyrosine(µmol/L)	**0.0001****
19	E-selectin(ng/ml)	**0.0004****
20	VEGF-A(pg/ml)	**0.0007****
21	Endothelin(pg/ml)	**0.0001****
22	Nitric oxide(µmol/L)	**0.0005****

UACR-Urine Albumin -Creatinine ratio; FBS-Fasting blood sugar(glucose);PPBS-Post prandial blood sugar(glucose); HbA1c- Glycated Hemoglobin; HOMA-IR- Homeostatic model assessment for Insulin resistance; HOMA-β- Homeostatic model assessment – Beta; QUICKI-Quantitative Insulin-Sensitivity Check Index; eGFR- Estimated Glomerular Filtration Rate; HDL-C—High Density Lipoprotein; LDL-Low Density Lipoprotein;VEGF-A-Vascular Endothelial Growth Factor A

P<0.05 – Significant and P<0.001 – highly significant**

The results pertaining to the mean and standard deviation of the data belonging to the various study groups in terms of the biochemical parameters are depicted in Table(6-12) and also portray the comparison of all the groups for ascertaining statistical significance of the various parameters, when examined with various test groups of nephropathy. Tables 6-12 show the data for NT, E-selectin and other biochemical parameters possessing different magnitude of statistical significance. A pronounced difference was observed in macro and microalbuminuric diabetic patients in comparison to normoalbuminuric diabetic patients.

The findings in the above data which demonstrate the diagnostic importance of nitric oxide in the context of imminent nephropathymake it clear that nitric oxide and albumin/creatinine ratio have a substantial association. There was also a significant association concerning Nitric oxide, insulin sensitivity, and IR.

The above analysis also confirms the diagnostic significance of VEGF-A and Endothelin in the context of impending nephropathy. Furthermore,the two biomarkers strongly correlate with albumin/creatinine ratio. Additionally, there is a strong association between VEGF-A, Endothelin, and insulin sensitivity, IR as determined by the established markers.

Two-way ANOVA test(Performed with the following dependent and independent variables):

The two-way ANOVA test was employed to determine the relationship between tworelated (associated) variables vis-a-vis dependent variable. The two-way ANOVA test would confer the benefit of arriving at an attenuated possibility of acquiringtype 1 errors, which would otherwise corrupt the data collected. The two-way ANOVA compares the means and variances among thevarious groups.

The dependent variables employed in the study are as follows. These variables denote measures of insulin sensitivity/insulin resistance: -

1.HOMA-IR

2.HOMA-Beta

3.TAG/HDL ratio

4. Triglyceride Glucose Index (TyG index)

The related variables taken up for comparison with the dependent variable(s) are depicted as given under: -

1.Nitro tyrosine and E selectin

2.Total cholesterol and LDL cholesterol

3.Total cholesterol and HDL cholesterol

4.FBS and HbA1c

5.Microalbuminuria and eGFR

6.UACR and eGFR

Table-13: Analysis of Variance (ANOVA)-Two way

Dependent variable	Related variables	"p"value		
		Normo albuminuria	Micro albuminuria	Macro albuminuria
HOMA-IR	Nitrotyrosine and E-Selectin	**0.0245***	**0.0174***	**0.0083***
	Total Cholesterol and LDL Cholesterol	0.0764	0.0532	0.0541
	Total Cholesterol and HDL Cholesterol	0.0675	**0.0471***	**0.0458***
	FBS and HbA1C	**0.0232***	**0.0167***	**0.0085***
	Microalbumin and e-GFR	-----	**0.0126***	**0.0086***
	UACR and e-GFR	-----	**0.0462***	**0.0075***
HOMA-β	Nitrotyrosine and E-Selectin	**0.0457***	**0.0264***	**0.0182***
	Total Cholesterol and LDL Cholesterol	0.0621	0.0532	0.0514
	Total Cholesterol and HDL Cholesterol	0.0787	0.0653	0.0532
	FBS and HbA1C	0.0522	**0.0484***	**0.0211***
	Microalbumin and e-GFR	-----	**0.0342***	**0.0113***
	UACR and e-GFR	-----	**0.0464***	**0.0274***
TAG/HDL Ratio	Nitrotyrosine and E-Selectin	0.0873	**0.0454***	**0.0242***
	Total Cholesterol and LDL Cholesterol	0.0786	0.0632	0.0632
	Total Cholesterol and HDL Cholesterol	0.0845	0.0764	0.0795
	FBS and HbA1C	0.0772	0.0522	**0.0414***
	Microalbumin and e-GFR	------	0.0563	**0.0461***
	UACR and e-GFR	------	0.0627	0.0582
Triglyceride Glucose Index	Nitrotyrosine and E-Selectin	0.0732	0.0581	**0.0494***
	Total Cholesterol and LDL Cholesterol	0.0856	0.0664	0.0562
	Total Cholesterol and HDL Cholesterol	0.0763	0.0541	**0.0443***
	FBS and HbA1C	0.0641	0.0576	**0.0357***
	Microalbumin and e-GFR	------	0.0547	**0.0395***
	UACR and e-GFR	------	**0.0487***	**0.0361***

UACR-Urine Albumin -Creatinine ratio; FBS-Fasting blood sugar(glucose); HbA1c- Glycated Hemoglobin; HOMA-IR- Homeostatic model assessment for Insulin resistance; HOMA-β- Homeostatic model assessment – Beta; QUICKI-Quantitative Insulin-Sensitivity Check Index; eGFR- Estimated Glomerular Filtration Rate; TAG-Triacyl glycerol; HDL-C—High Density Lipoprotein; LDL-Low Density Lipoprotein

P<0.05 – Significant* P<0.01 – Significant**

The dependent variables (all related to insulin resistance/insulin sensitivity), namely HOMA-IR, HOMA-β, TAG/HDL and TyG index were specifically chosen to assess the relationship with the variables (in related pairs). Our results show very good association when the dependent variables were analyzed in the light of the magnitude of impending nephropathy, with respect to related pairs, namely Nitrotyrosine and E-Selectin; Total Cholesterol and LDL Cholesterol; Total Cholesterol and HDL Cholesterol; FBS and HbA1C; Microalbumin and e-GFR; UACR and e-GFR. The relationship denotes the diagnostic relevance of the comparison of related independent variables with the individual, dependent variable signifying insulin sensitivity/insulin relevance.

Table-14: Correlation among specific parameters(Nitrotyrosine,E-selectin,VEGF-A,Endothelin,Nitric oxide) with other biochemical parameters:

	Correlation Coefficient (r)				
Parameter	**Nitrotyrosine**	**E-selectin**	**VEGF-A**	**Endothelin**	**Nitric oxide**
UACR	**0.587****	**0.674****	**0.823****	**0.847****	**0.718****
FBS	0.314*	0.442*	0.401*	0.388*	0.491*
PPBS	0.347*	0.376*	0.397*	0.402*	0.448*
Insulin	**0.512****	0.498*	0.441*	0.427*	0.467*
HbA1C	0.425*	**0.598****	0.498*	**0.568****	**0.521****
HOMA-IR	**0.595****	**0.687****	**0.864****	**0.943****	**0.751****
HOMA BETA	**-0.664****	**-0.664****	**-0.734****	**-0.736****	**-0.585****
QUICKI	**-0.532****	**-0.732****	**-0.846****	**-0.921****	**-0.774****
eGFR	**-0.527****	**-0.527****	**-0.527****	**-0.512****	-0.466*
Total cholesterol	0.235	0.265*	0.343*	0.277*	0.332*
Triglycerides	0.247	0.283*	0.276*	0.231*	0.298*
HDL-C	-0.227	-0.278*	-0.291*	-0.246*	-0.276*
LDL-C	0.173	0.201*	0.206*	0.253*	0.275*
Urea	0.213*	0.304*	0.275*	0.288*	0.289*
Creatinine	0.341*	0.462*	0.298*	0.301*	0.266*

UACR-Urine Albumin -Creatinine ratio; FBS-Fasting blood sugar(glucose);PPBS-Post prandial boood sugar; HbA1c- Glycated Hemoglobin; HOMA-IR- Homeostatic model assessment for Insulin resistance; HOMA-β- Homeostatic model assessment – Beta; QUICKI-Quantitative Insulin-Sensitivity Check Index; eGFR- Estimated Glomerular Filtration Rate;HDL-C--High Density Lipoprotein-cholesterol; LDL-C--Low Density Lipoprotein cholesterol

****Correlation is significant at the 0.01 level (2-tailed).*Correlation is significant at the 0.05 level (2-tailed)**

Glycemic index:

Mean of FBS(mg/dl) was 83.96± 5.36 in control, whereas in thenormoalbuminuria group it was 135.12± 16.29; in microalbuminuria group, the mean of FBS was 149.5± 27.98; macroalbuminuria group depicted a mean for FBS as 252.4± 33. Plasma glucose in PPBS(mg/dl) state in the Control group (mean) was 1112.3± 11.12; in the normoalbuminuria group, the mean was 179.78± 19.08; in the microalbuminuria group, the mean was 209.2± 38.33 and in the macroalbuminuria group, the mean was 331.8 ±30.1 'p' value less than 0.05 was considered statistically significant. FBS/ PPBS and the degree of renal damage were positively correlated.

HbA1C or glycated Hemoglobin(%)less than 6% was considered as normal or non -diabetic. In the present study, we had reported a mean for HbA1C in the control group as 5.78± 0.55, in normoalbuminuria group, the average mean was 7.55± 0.84, in microalbuminuria group , the average mean was 8.45± 0.99 and in macroalbuminuria group it was 9.53± 0.49. There was a gradual increase in the value of HbA1C, which indicates poor control or uncontrolled diabetes mellitus. Whereas, in the control group, HbA1C value was within normal limits i.e. less than 6%. High numerical number indicates poor prognosis. 'p' value less than 0.05 was considered statistically significant. HbA1C and the degree of renal damage was positively correlated.

Lipid profile:

The mean of serum Total cholesterol(mg/dl) was 169± 9.23 in the control group; The mean of normoalbuminuria group was 189.08± 18.3, where as the mean in the microalbuminuria groupwas 197.66± 23.47; and mean in the macroalbuminuria group was 209.64± 21.73. 'Total cholesterol levels were moderately elevated in diabetic nephropathic cases when compared to the control.

Triglycerides(mg/dl) in the control group 98.3±7.22, in normoalbuminuria group, the mean was 119.1± 28.64, mean in microalbuminuria group was 136.86± 28.63 and in macroalbuminuria group 162.66± 16.7. Plasma triglycerides were elevated and positively correlated with diabetic nephropathy. The values were statistically significant.

Serum HDL-cholesterol(mg/dl) showed a mean of 42.72± 2.65 in the control group; in normoalbuminuria, the group mean was 39.38± 3.0; in the microalbuminuria group, the mean was 38.62 ± 2.13, whereas in the macroalbuminuria group, the mean was 36.36 ± 3.0. There was a negative correlation between HDL-cholesterol and diabetic nephropathy and the 'p' values were statistically significant.

The mean of serum LDL –Cholesterol(mg/dl) was 106.62± 10.22 in control group; Mean in normoalbuminuria group was 125.88± 14.82; Mean in microalbuminuria group was 131.67± 22.8 and mean in macroalbuminuria group was140.75± 21.74. LDL-cholesterol was positively correlated with diabetic

nephropathy. ‘p’ values were found to be less than 0.05 and hence statistically significant.

Urinary creatinine:

Urinary creatinine(mg/dl) depicted mean of 88.86± 4.06 in the control group which is within normal range; mean in normoalbuminuria group was 85.38± 9.43; mean in microalbuminuriawas 63.52± 19.28 and in the macroalbuminuria group it was 44.14± 6.86. Urinary creatinine levels decreased as the nephropathy progresses (from normoalbuminurics to macroalbuminurics). The values were statistically significant(‘p’ value less than 0.05). Urinary creatinine negatively correlated with diabetic nephropathy and statistically significant.

Micro albumin:

Microalbumin(mg/dl) excretion is the index of renal function. In the present research study we found in the control a mean that corresponded to 16.94± 1.58, In thenormoalbuminuria group, the mean was 19.742 ± 2.83; in the microalbuminuria, the group mean was 77.72± 11.66, whereas in the macroalbuminuria, the group mean was 187.18± 20.85. In control group the microalbumin level was within the normal ranges. In micro albuminuric group, it wasslightly elevated. In the microalbuminuricgroup, it was moderately elevated. In Macroalbuminuria, urinary microalbumin was highly elevated. Urinary microalbumin levelspositively correlated with diabetic nephropathy. The ‘p’ values less than 0.05 was considered significant.

Urinary albumin creatinine ratio(UACR):

Albumin creatinine ratio mean in the control group was 19.1± 1.94, mean in normoalbuminuria group was 23.27± 3.4, mean in the microalbuminuria group 132.51± 38.57; whereas the mean in Macroalbuminuriagroup was430.08± 55.4. Albumin creatinine ratios were significantly elevated when compared with control group.’p’ value less than 0.05 was considered statistically significant.

Blood urea and serum creatinine:

In the present study, the mean of blood urea(mg/dl) in the control group was 25.12± 3.94, in normoalbuminuria group 27.78± 4.07; whereas in themicroalbuminuria group it was 31.66± 4.21 and in the macroalbuminuria group

31.48± 2.63. The means of all groups for blood urea was within the normal limits. The mean of serum creatinine (mg/dl) level in the control group was 0.73± 0.17, in normoalbuminuriait was 0.78± 0.24, microalbuminuria group had a mean 0.97± 0.31 and in the macroalbuminuria group 1.08± 0.29.

Plasma Insulin:

The mean of plasma insulin(µIu/ml) in control group was 6.66± 0.58, Mean in normoalbuminuria group was10.67± 2.65; whereas mean in the microalbuminuriagroup was 15.3± 3.18 and that in macroalbuminuria group 18± 2.27 . Plasma insulin levels had gradually increased from normoalbuminurics to macroalbuminuricand a positive correlation was obtained between diabetic nephropathy and plasma insulin levels.

HOMA-IR

The mean of HOMA - IR level in control group is about 1.38± 0.14, in normoalbuminuria group mean is 3.59± 1.07, mean in microalbuminuria group is about 5.69± 1.68 and in macroalbuminuria group mean is about 11.25± 2.13 . IR is significantly elevated in all groups except in control group. There is a positive correlation is seen with diabetic nephropathy. 'p' values are less than 0.05 and statistically significant. HOMA – IR level positively correlating the hyperglycemia.

HOMA - BETA:

Mean of HOMA - BETA level in control group is about 122.62± 35.26, in normoalbuminuria group mean is 55.69± 18.8, mean in microalbuminuria group is about 46.35± 17.88 and in macroalbuminuria group mean is about 35.39± 6.75 . IR is significantly elevated in all groups except in control group. There is a positive correlation is seen with diabetic nephropathy. 'p' values are less than 0.05 and statistically significant.

QUICKI

Mean of QUICKI level in control group is about 0.36 ± 0.01, in normoalbuminuria group mean is 0.32± 0.01, mean in microalbuminuria group is about 0.3± 0.02 and in macroalbuminuria group mean is about 0.27± 0.01 . IR is significantly elevated in all groups except in control group. There is a positive

correlation is seen with diabetic nephropathy. ‘p’ values are less than 0.05 and statistically significant.

eGFR:

Mean of eGFRlevel in control group is about 116.01± 16.68, in normoalbuminuria group mean is 108.98± 29.69, mean in microalbuminuria group is about 85.9± 24.11 and in macroalbuminuria group mean is about 79.38± 21.79 . IR is significantly elevated in all groups except in control group. There is a positive correlation is seen with diabetic nephropathy. ‘p’ values are less than 0.05 and statistically significant.

Nitrotyrosine:

In the present study mean of serum Nitrotyrosine(µmol/L)in control group are 0.21 ± 0.03, mean in normoalbuminuria group is about 0.28 ± 0.05, mean in microalbuminuria group is about 0.36 ± 0.1 and in macroalbuminuria group mean is 0.5 ± 0.1. Serum Nitrotyrosine levels are significantly elevated compared with control group. Positive correlation is seen with diabetic nephropathy. ‘p’ values are < 0.05, statistically significant.

E-selectin:

In the present study mean of serum E-selectin(ng/ml) in control group are 23.12 ± 3.81, mean in normoalbuminuria group is about 35.38 ± 6.46, mean in microalbuminuria group is about 67.74 ± 8.69 and in macroalbuminuria group mean is 91.76 ± 7.31. Serum E-selectin levels are significantly elevated compared with control group. Positive correlation is seen with diabetic nephropathy. ‘p’ values are < 0.05, statistically significant.

VEGF-A:

In the present study mean of serum VEGF-A(pg/ml) in control group are 75 ± 7.21, mean in normoalbuminuria group is about 119.5 ± 16.66, mean in microalbuminuria group is about 139.13 ± 14.5 and in macroalbuminuria group mean is 173.38 ± 10.32. Serum VEGF-A levels are significantly elevated compared with control group. Positive correlation is seen with diabetic nephropathy. ‘p’ values are < 0.05, statistically significant.

Endothelin:

In the present study mean of serum Endothelin(pg/ml)in control group are1.39 ± 0.08, mean in normoalbuminuria group is about 2.7 ± 0.56, mean in microalbuminuria group is about 3.51 ± 0.3 and in macroalbuminuria group mean is 5.11 ± 0.49. Serum Endothelin levels are significantly elevated compared with control group. Positive correlation is seen with diabetic nephropathy. 'p' values are < 0.05, statistically significant.

Nitric oxide:

In the present study mean of serum Nitric oxide(µmol/L) in control group are 33.36 ± 3.24, mean in normoalbuminuria group is about 42.64 ± 6.14, mean in microalbuminuria group is about 45.9 ± 7.03 and in macroalbuminuria group mean is 58.56 ± 10.82. Serum Nitric oxide levels are significantly elevated compared with control group. Positive correlation is seen with diabetic nephropathy. 'p' values are < 0.05, statistically significant.

The mean values are represented in the Bar Graphs, for the sakeof clarity and easy reference (Fig. 1-11)

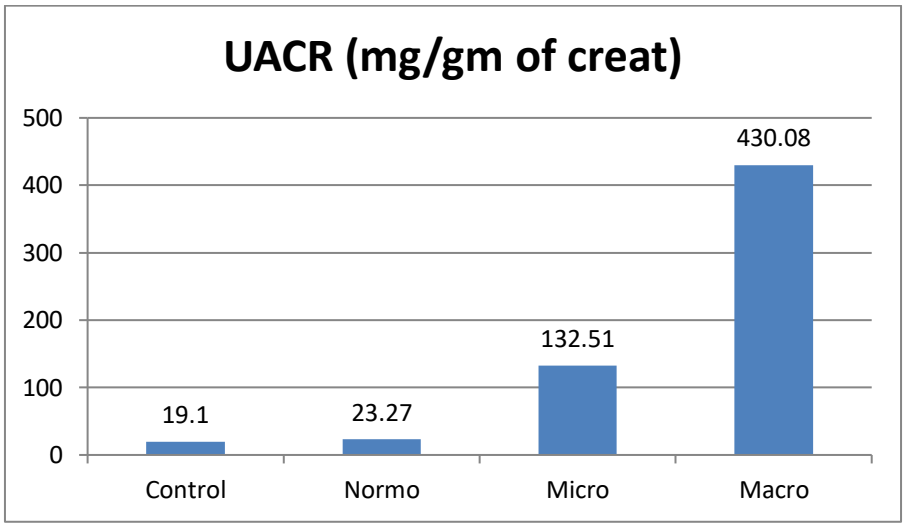


Figure-1: UACR

UACR-Urine Albumin -Creatinine ratio

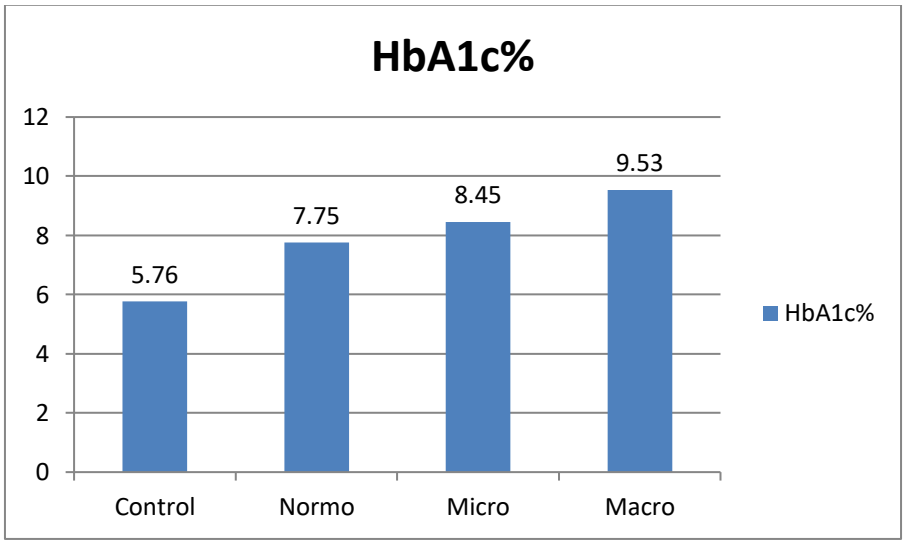


Figure-2: HbA1c%

HbA1c- Glycated Hemoglobin

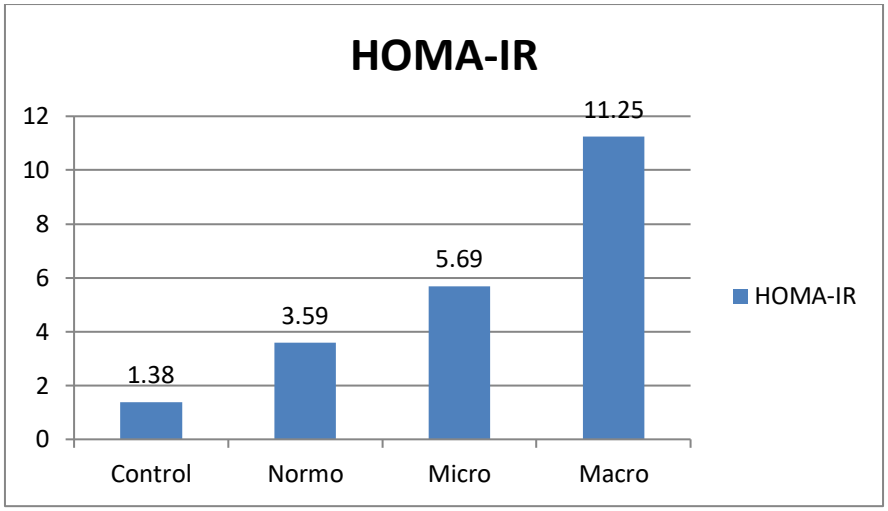


Figure-3: HOMA –IR

HOMA - IR- Homeostatic model assessment for Insulin resistance

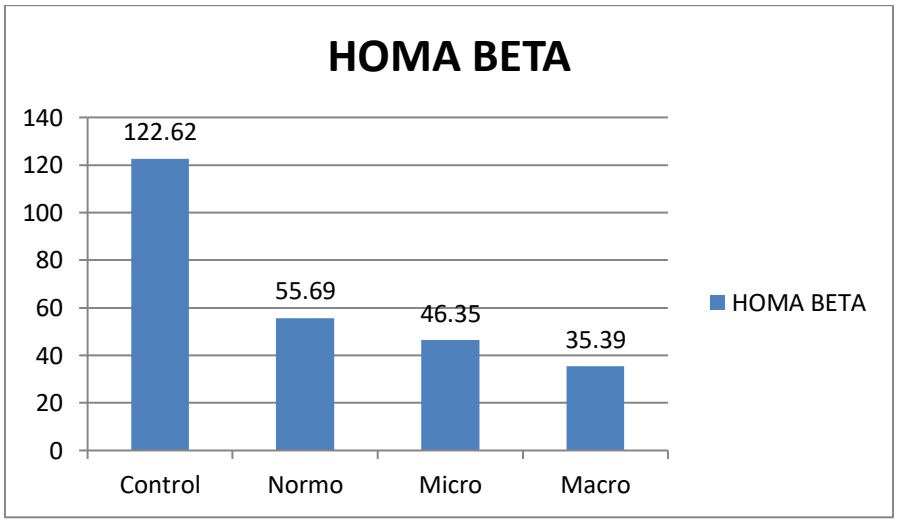


Figure-4: HOMA BETA

HOMA-β- Homeostatic model assessment – Beta

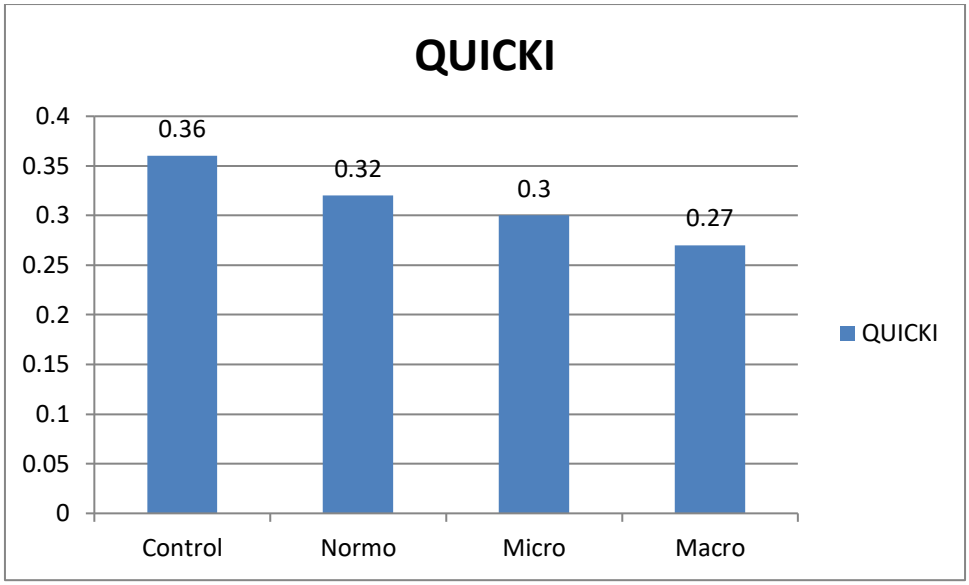


Figure-5: QUICKI

QUICKI-Quantitative Insulin-Sensitivity Check Index

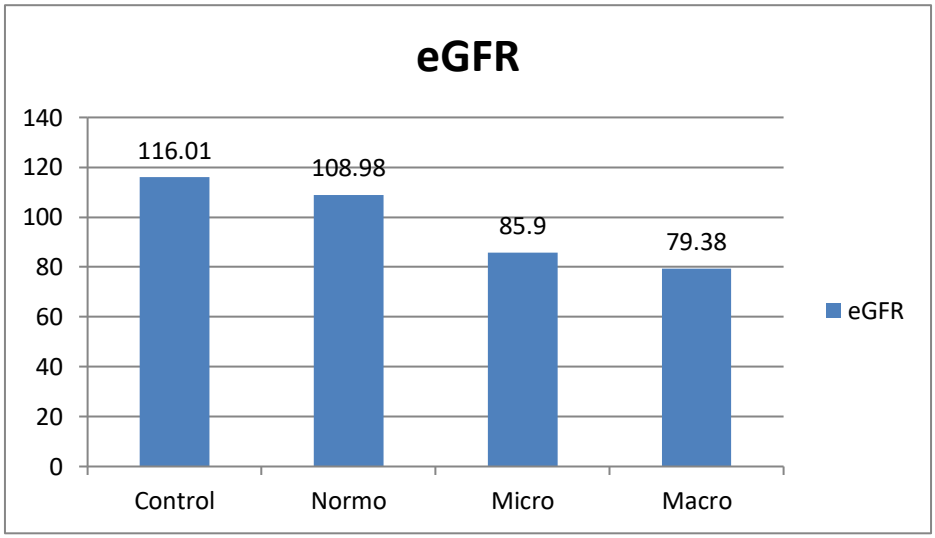


Figure-6: e-GFR

eGFR- Estimated Glomerular Filtration Rate

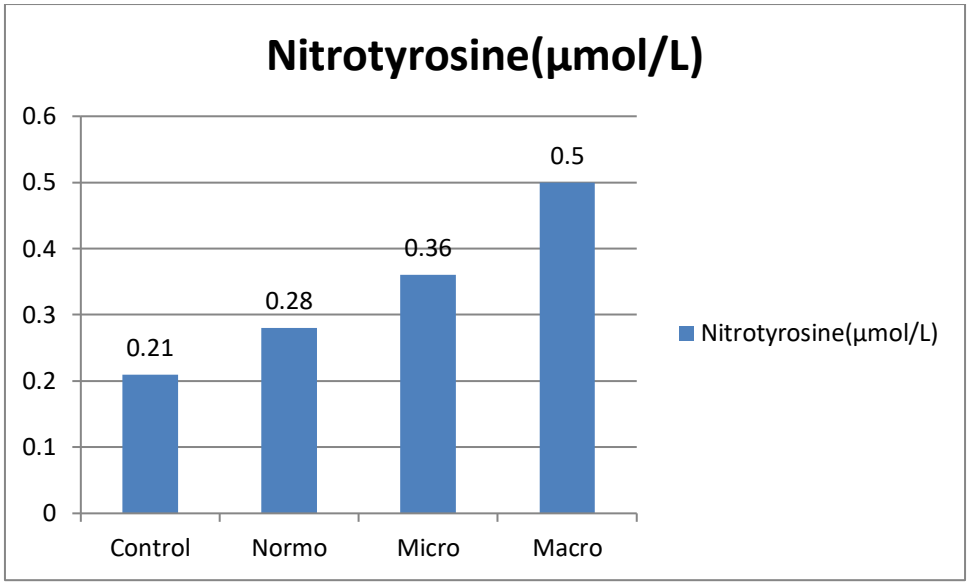


Figure-7: Nitrotyrosine

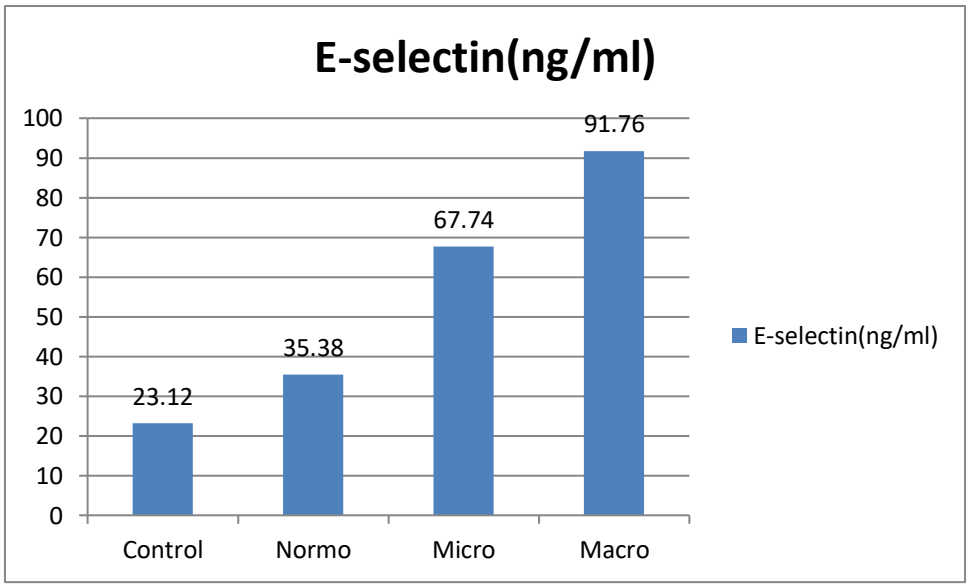


Figure-8: E-Selectin

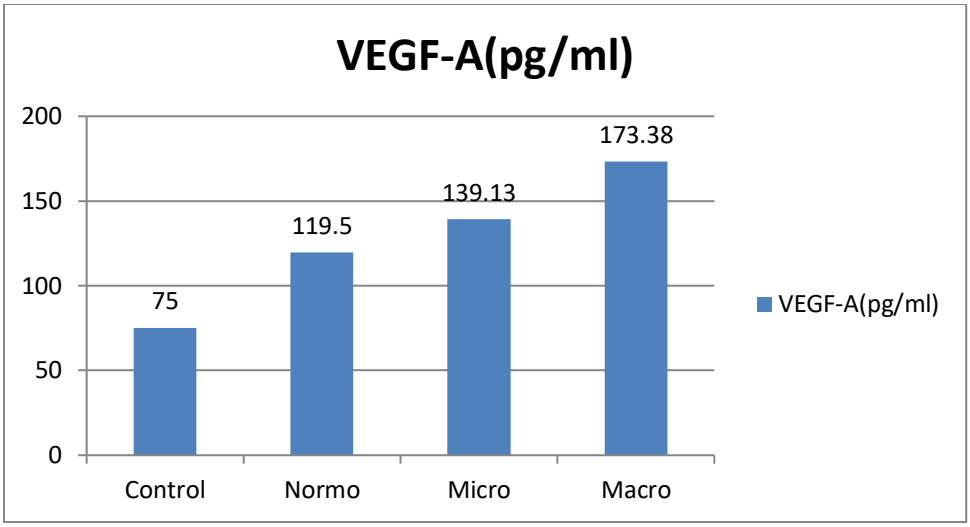


Figure-9: VEGF-A

VEGF-A-Vascular Endothelial Growth Factor A

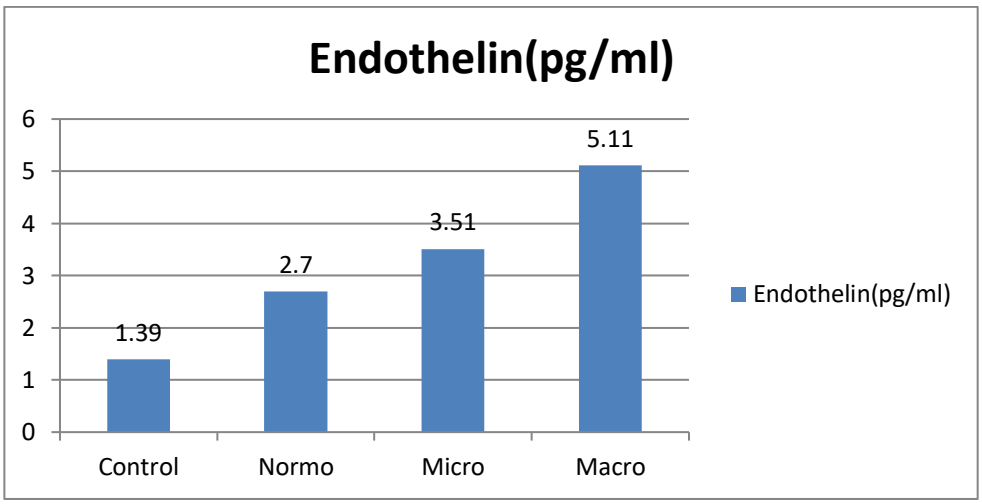


Figure-10: Endothelin

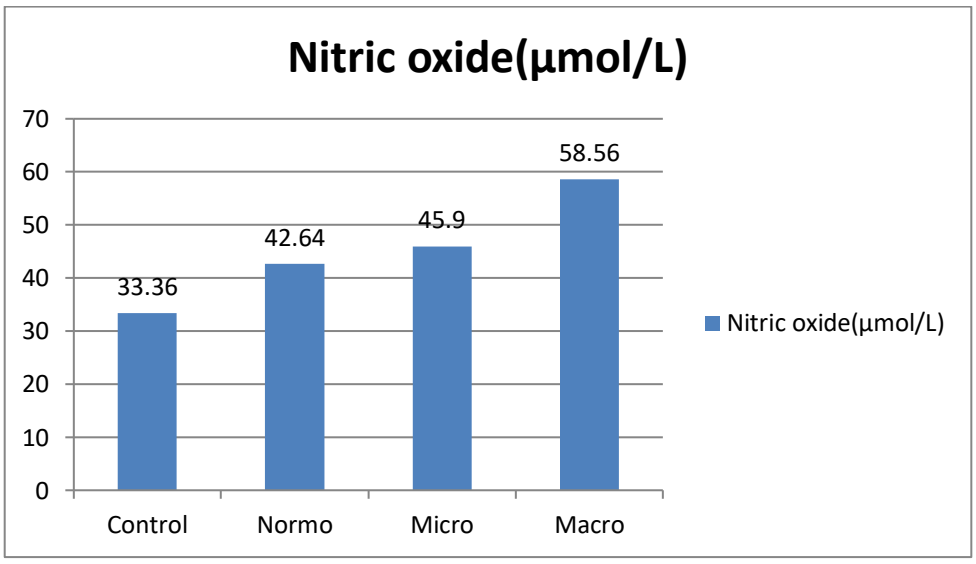


Figure-11: Nitric Oxide

Receiver Operating Characteristic (ROC) curves

ROC curves were elaborated based on the sensitivity and specificity with reference to the independent variable, namely NT of normoalbuminuria, microalbuminuria and macroalbuminuria groups as against HOMA – IR (insulin resistance), the dependent variable. We analyzed the data accordingly as Control vs. Normoalbuminuria (Fig.12), Control vs Microalbuminuria (Fig13), and Control vsMacroalbuminuria (Fig.14). The results clearly indicate that NT could be used as a predictor of impending vascular complications in diabetic nephropathy, in the light of albuminuria.

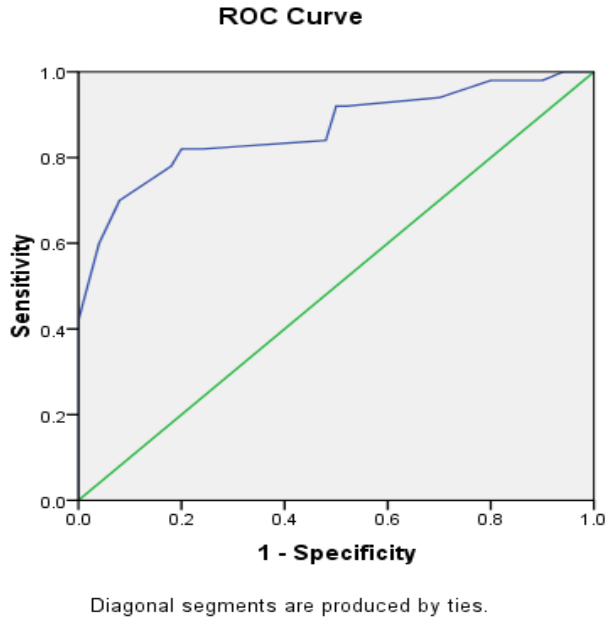


Fig.12-ROC: ControlvsNormoalbuminuria

Area Under Curve (AUC)=0.865; Confidence interval=0.793-0.938; Standard error-0.037;Sensitivity=0.800; specificity= 0.820; Cut-off (miniimum) =0.245

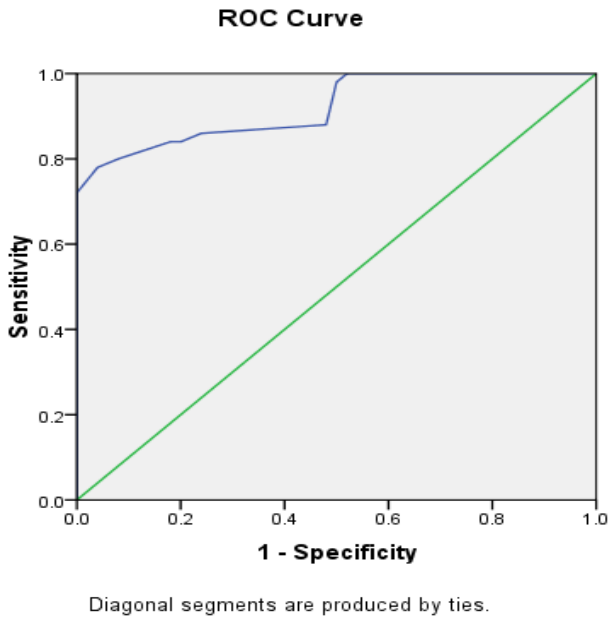


Fig.13-ROC: ControlvsMicroalbuminuria

Area Under Curve (AUC) =0.922; Confidence interval =0.870 – 0.974; Standard Error=0.027; Sensitivity= 0.920; Specificity= 0.800; Cut-off (minimum)=0.265

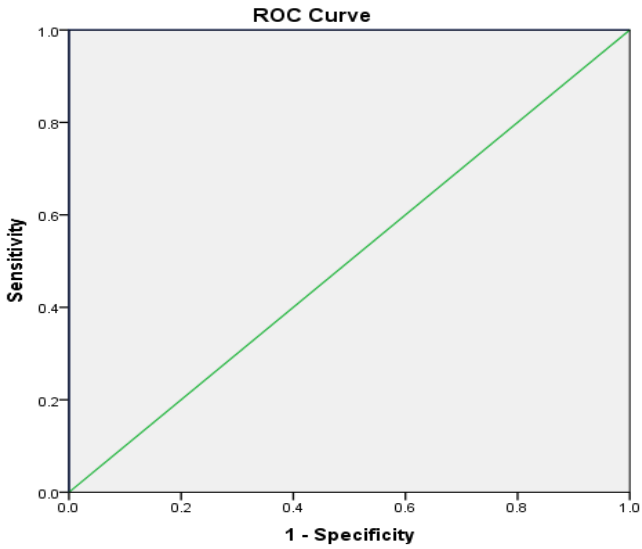


Fig.14-ROC: ControlvsMacroalbuminuria

Area Under Curve (AUC) =1-000; Confidence interval =1-000-1-000; Standard Error=0.000; Sensitivity= 1.000; Specificity= 1.000; Cut-off (minimum)=0.290

Inference: Nitro Tyrosine could function as a predictor, with reference to the magnitude of albuminuria

Scatter plots :

The scatter plots(Fig 15-22)which indicate the association between the two variables and scatter plot matrix show all the pairwise scatter plots for the variables.

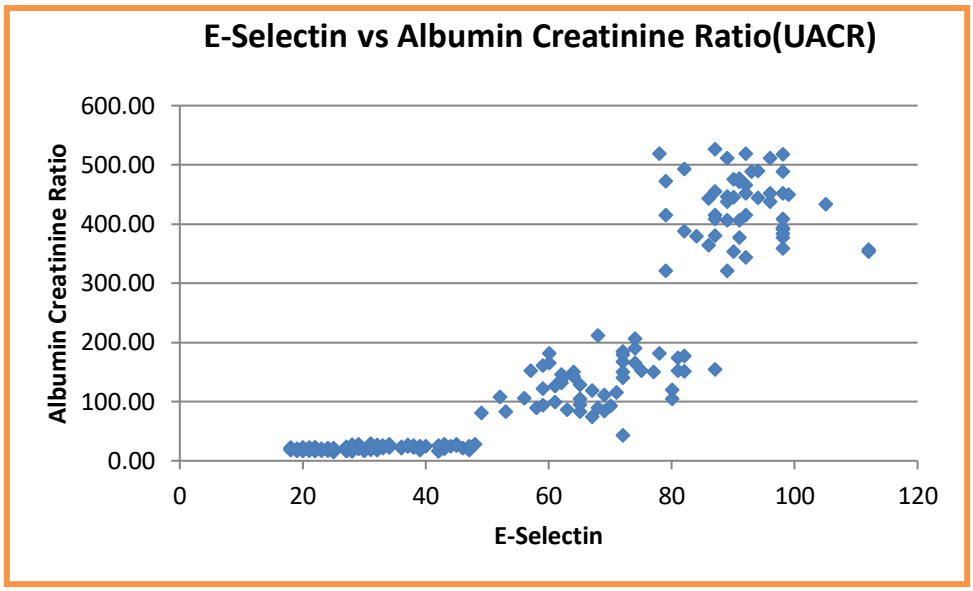


Fig.15: Scatter Plot of E-Selectinvs Albumin Creatinine Ratio(UACR)

UACR-Urine Albumin -Creatinine ratio

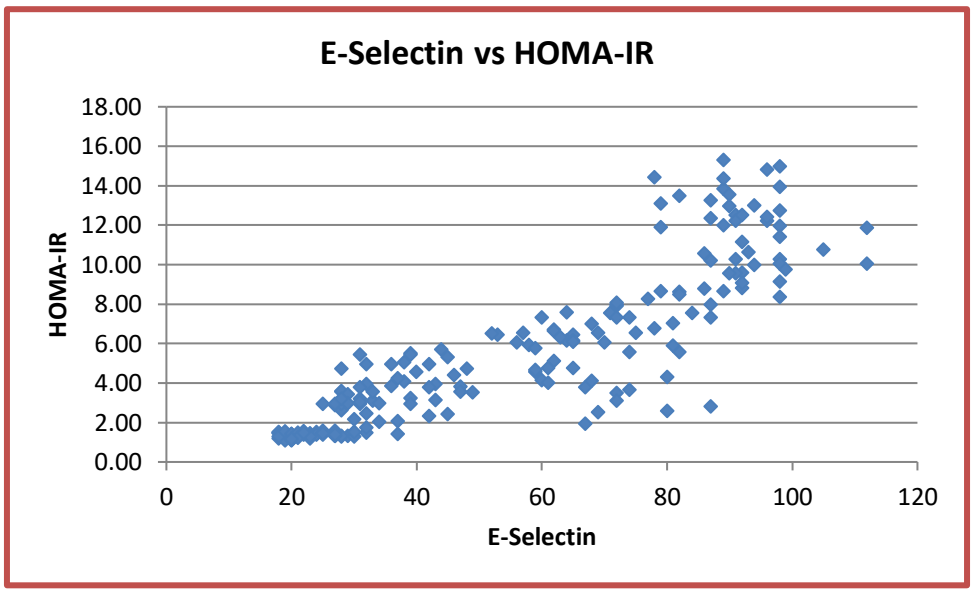


Fig.16: Scatter Plot of E-Selectinvs HOMA-IR

HOMA-IR- Homeostatic model assessment for Insulin resistance

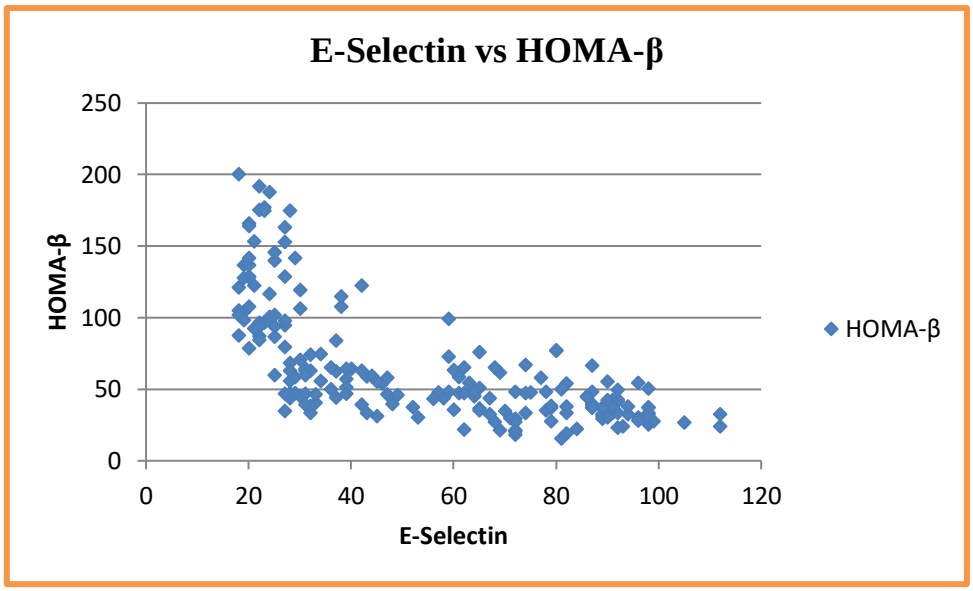


Fig.17: Scatter Plot of E-Selectinvs HOMA-β

HOMA-β- Homeostatic model assessment – Beta

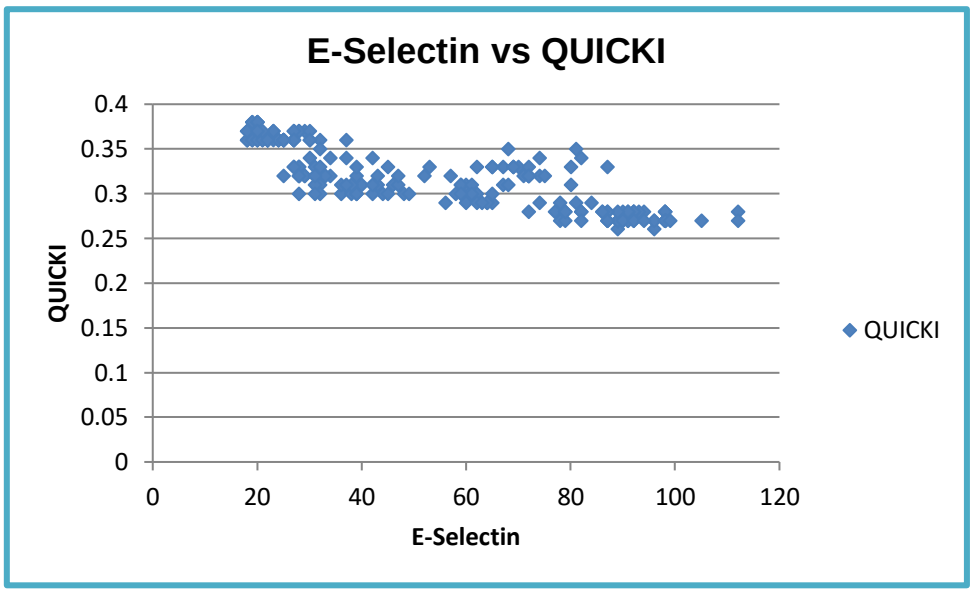


Fig.18: Scatter Plot of E-Selectinvs QUICKI

QUICKI-Quantitative Insulin-Sensitivity Check Index

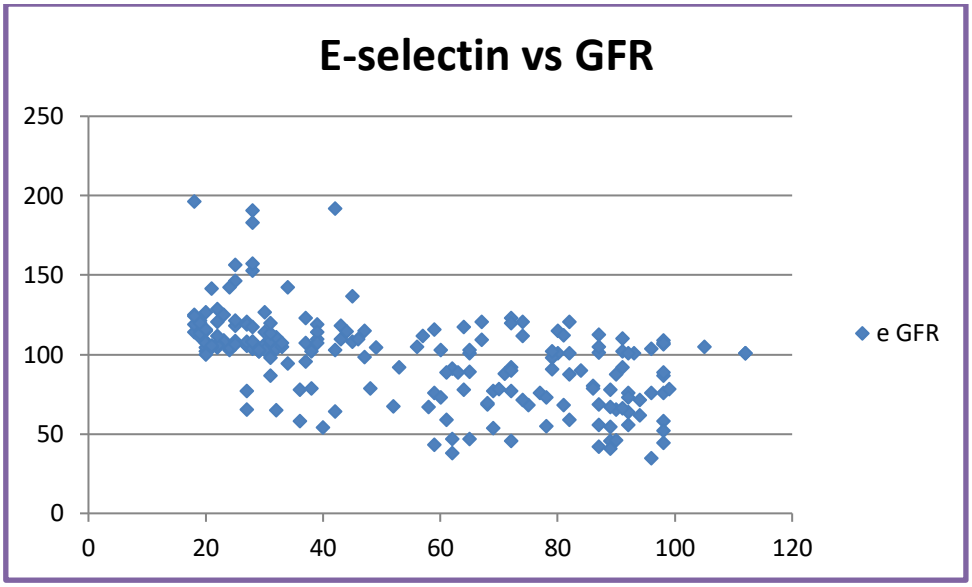


Fig.19: Scatter Plot of E-SelectinvseGFR

eGFR- Estimated Glomerular Filtration Rate

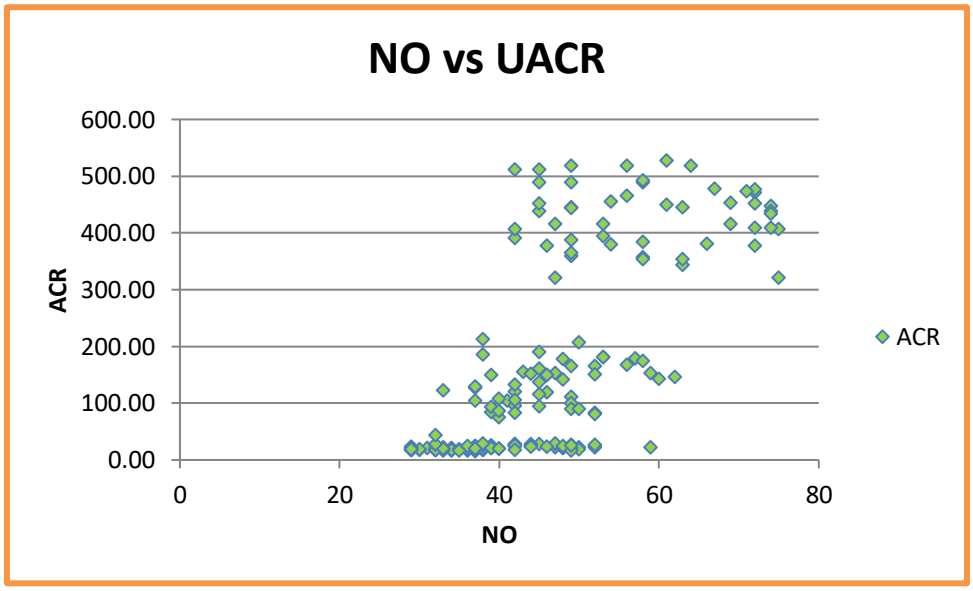


Fig. 20: Scatter plot of NOvsAlbumin Creatinine Ratio(UACR)

NO-Nitric oxide;UACR-Urine Albumin -Creatinine ratio

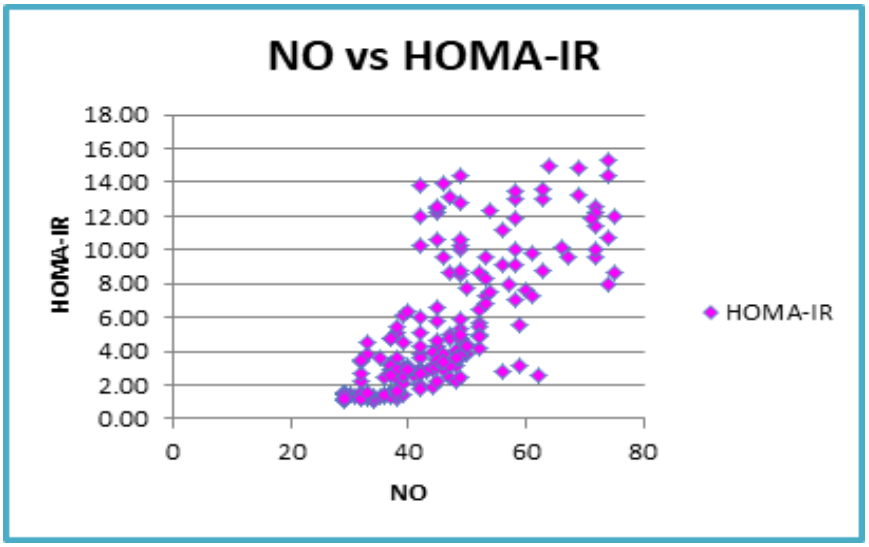


Fig. 21: Scatter plot of NO Vs HOMA-IR

NO-Nitric oxide;HOMA-IR- Homeostatic model assessment for Insulin resistance

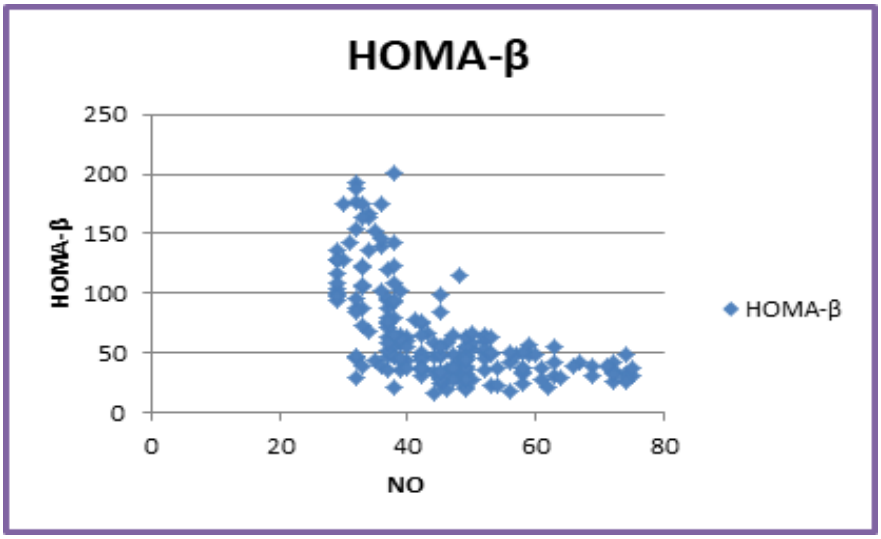


Fig.22: Scatter plot of NO vs**HOMA-β**

NO-Nitric oxide;HOMA-β- Homeostatic model assessment – Beta

The above-mentioned scatter plots(Fig.15-19) denote those between E-selectin and other parameters(UACR,HOMA-IR,HOMA-Beta,QUICKI,eGFR). With reference to the plot enabled between E-selectin and UACR,HOMA-IR, the pattern follows an increasing trend and hence the association is positive. The plot between E-selectin and HOMA-Beta,QUICKI,eGFR follows a decreasing trend, so the correlation is negative. Scatter plots(Fig.20-22) denote those between NO and other parameters(UACR,HOMA-IR,HOMA-Beta).The trend between NO and UACR,HOMA-IR, follows a positive pattern. The trend between NO and HOMA-Beta is negative.

9. DISCUSSION

The imbalance between the prooxidants and antioxidants in the human body, as exemplified by the magnitude of the state of oxidative stress culminates in cellular disruption and damage (58). The free-radicals are having a lone electron in the outer orbital and by virtue of its reactive nature act on polyunsaturated fatty acids and other biomolecules including carbohydrates and proteins.

Lipid peroxidation products include conjugated dienes and malondialdehyde (MDA) that are increased in T2DM patients . In the current study, we observed that levels of Nitrotyrosine (NT), a product of free radical induced protein modification were significantly increased in T2DM subjects. A pronounced difference was observed in macro and microalbuminuric diabetic patients in comparison to normoalbuminuric diabetic patients. Peroxynitrite, a chemical product formed by the reaction between superoxide and nitric oxide (NO) modifies tyrosine, an aromatic amino acid in proteins to form NT. NT is involved in the inactivation of mitochondrial and cytosolic proteins, synonymous with the damage inflicted on the various cellular constituents (59). Studies have revealed that chronic hyperglycemia, dyslipidemia and oxidative stress lead to inflammation by aggravating IR. Furthermore, an enhancement in the expression of adipokines and macrophage inflammatory proteins are implicated in macrophage infiltration, as a manifest in the adipose tissue. This eventually leads to an exaggerated production of reactive oxygen species (ROS) and inflammatory cytokines. It might be of contemporary interest and relevance as well to note that adipose tissue dysfunction could culminate in adipocyte-specific deletion of a redox-sensitive transcription factor binding to the designated promoter region of defined genes whose gene products are antioxidant enzymes (60).

In the present study,we had noticed pronouncedly high serum cholesterol, LDL and TAG in type 2 diabetics with reference to healthy controls. A positive association between NT and urine albumin -to-creatinine ratio (UACR), PPBS, glycemic control, insulin resistance, serum triacylglycerols and negative association with HDL -cholesterol were elicited from our study. The results emerging from the ROC analysis reveal the biochemical utility of NT as a reliable predictor of

impending vascular complications in nephropathy, as observed in insulin resistant type 2 diabetics, but with varying magnitude of albuminuria. However, very few studies are there citing the utility of an objective biomarker in predicting future vascular complications in nephropathy associated with insulin resistance. Hence, the present study strives to acquire significance in this context.

Hyperglycemia is pronouncedly synonymous with alterations in lipid metabolism which inflicts adverse effects typified by enhanced HDL clearance, decreased apoA-1 transcription and accelerated HDL glycation. These biochemical events predispose to atherosclerosis/arteriosclerosis. Oxidative stress continues to be the common nexus for the major biochemical pathways involved in the genesis and progression of diabetic micro- and macrovascular complications .

Nitrosative stress and peroxynitrite are not only implicated in diabetes-induced cardiovascular aberration, but also would contribute to the pathogenesis and progression of diabetic nephropathy. Accordingly, neutralization of peroxynitrite could afford a novel approach in the prevention and management of diabetic complications. Hence, it must be said that NT levels could be a novel biochemical predictor of nephropathy in type 2 diabetic patients, especially at a time when not many simple, economically viable, specific and sensitive predictors IR induced diabetic nephropathy are not available in the armamentarium (61).

In the present study, we observed that E-selectin levels were significantly increased in T2DM subjects that could be attributed to oxidative stress. Significant difference was observed in macro and microalbuminuric diabetic patients in comparison to normoalbuminuric diabetic patients. Moreover, in the present study, we made a sincere attempt to document E-selectin as an objective marker of oxidative stress. In view of this, we had eschewed other conventional markers of oxidative stress. It is well documented that the biochemical events, namely hyperglycemia and oxidative stress lead to inflammation and IR is regarded as central to this phenomenon. Thus, E-selectin levels could prove to be a putative and novel biomarker of nephropathy in type 2 diabetic patients, especially at a juncture when very few simple, economically viable, specific, and sensitive predictors of IR induced diabetic nephropathy are available in the existing armamentarium. (62). A pronounced

association between E-selectin and other parameters, namely UACR, HbA1C,HOMA-IR, HOMA BETA, QUICKI and eGFR was observed in our study. The results pertaining to ROC analysis also reveal the usefulness of E-selectin as a reliable predictor of impending nephropathy, a common complication observed in insulin resistant type 2 diabetics.

We had attempted to evaluate the relationship between the endothelial dysfunction markers VEGF-A and Endothelin-1 with reference to the IR and insulin sensitivity metrics, namelyHOMA-IR, HOMA- β, and QUICKI in nephropathy observed in T2DM patients, but with respect to gradation based on UACR. In the entire study population, HOMA-IR, HOMA-β, VEGF-A, and Endothelin-1 were the risk factors for diabetic nephropathy. However, noticed that the magnitude of HOMA-IR, VEGF-A, and Endothelin-1 were the risk factors for diabetic nephropathy in the micro- and macroalbuminuric nephropathy but not in the normoalbuminuric nephropathy. It may please be recalled that the subjects were segregated into three groups on the basis of urinealbumin-to-creatinine ratio (UACR). This study findings depict a strong correlation among the levels of insulin resistance, VEGF-A, and endothelin-1 with the decline in diabetic kidney function.Studies have shown as to how HOMA-IR, VEGF-A, and Endothelin-1 could contribute to diabetic nephropathy(63).However, it is not unequivocal.

A previous report highlighted the association between IR (HOMA-IR) and kidney dysfunction and that HOMA-IR levels were higher in all three stages in diabetic nephropathy namely Normoalbuminuria, Microalbuminuria, and macroalbuminuria. HOMA-IR was correlated with eGFR and UACR. An indicator of insulin secretory capacity, the homeostasis model assessment of β -cell function (HOMA- β) is derived from fasting plasma glucose and insulin concentrations. It has been used in the evaluation ofβ -cell failure in T2DM brought about by low-grade inflammation and oxidative stress in the pancreas, as well as to predict the onset of diabetes in non-diabetics. According to the evidences generated in the present study, β -cell dysfunction is a risk factor for renal illness. Decreased HOMA- β values in diabetic nephropathy was noted, besides eGFR and UACR that had inverse relationships with HOMA- β, as per a previous report. These findings suggest that HOMA- β could contribute to the elevated risk of proteinuria independently of

diabetic nephropathy, despite the strong relationship between pancreatic β -cell failure and diabetic nephropathy. Fasting blood sugar and insulin levels are transformed mathematically to create the quantitative insulin-sensitivity check index (QUICKI)(64).

The findings of the present study indicate that IR enhances the risk of kidney disease. In diabetic nephropathy, QUICKI readings were lower. QUICKI is considered as an indirect marker of insulin resistance, and studies have indicated that it plays a role in the development of diabetic nephropathy. eGFR and UACR demonstrate inverse associations with QUICKI.Inflammation, oxidative stress, and enhanced lipotoxicity, which culminate in the development of microangiopathy, are potential mechanistic avenues that link IR to diabetic nephropathy, even though the mechanism behind the linkage is yet to be comprehensively elucidated. Numerous studies have revealed that dyslipidemia plays a significant part in the development of renal damage in people with diabetes. Despite the significant association between Insulin receptor sensitivity and diabetic nephropathy, these results suggest that QUICKI could contribute to the enhanced risk of proteinuria, independent of diabetic nephropathy. Vascular endothelial growth factor (VEGF-A) is an important player in vasculogenesis and neoangiogenesis and promotes cell proliferation. Other actions include inhibition of apoptosis, enhanced inflammation. Endothelial dysfunction is influenced by insulin resistance, which is nothing but a form of systemic low-grade inflammation. The type-2 diabetes mellitus patients exhibited higher VEGF-A levels and a higher likelihood of renal dysfunction(65). By activating the VEGFR2-nephrin-nck-actin signaling cascade in podocytes, chronic hyperglycemia-induced excess podocyte VEGF-A and low endothelial nitric oxide promote the onset and progression of diabetic nephropathy.The abnormal cross-talk between the VEGF-A and NO pathways is propelled by the elevated oxidative stress(66) this results in podocyte permeability.

Three isotypes of endothelin ET-1, ET-2, and ET-3 are regarded as powerful vasoactive peptides that have recently been linked to diabetes. In this study, elevated ET-1 levels were discovered in all three stages of diabetic patients. This finding, in conjunction with the highly correlated HOMA-IR and HOMA- parameters measuring IR and beta cell dysfunction as well as the QUICKI insulin receptor sensitive index,

demonstrate the high risk of nephropathy associated with type-2 diabetes and endothelial dysfunction.It must be stated that ET-1 is a crucial biomarker for endothelial dysfunction, which is probably well connected to the free radicals observed in diabetes(67). Our work is one among the few instances to compare the various phases of nephropathy in type -2 diabetes mellitus with insulin resistance, VEGF-A, and ET-1.

10. SUMMARY & CONCLUSION

In the current study, we observed that levels of Nitrotyrosine (NT),a product of free radical induced protein modification were significantly increased in T2DM subjects.A pronounced difference was observed in macro and microalbuminuric diabetic patients in comparison to normoalbuminuric diabetic patients. The results emerging from the ROC analysis reveal the biochemical utility of NT as a reliable predictor of impending vascular complications in nephropathy as observed in insulin resistant type 2 diabetics. A positive association between NT and urine albumin-to-creatinine ratio(UACR), PPBS, glycemic control, insulin resistance,serum triacylglycerols and negative association with HDL - cholesterol were elicited from our study. Hence the biochemical assessment of Nitrotyrosine could be useful to predict the progression of diabetic nephropathy as a biomarker as a function of UACR and e-GFR.

A pronounced association between E-selectin and other parameters, namely UACR, HbA1C,HOMA-IR, HOMA BETA, QUICKI and eGFR was observed in our study. It is concluded that the biochemical assessment of E-selectin could predict the progression of nephropathy, a major complication in type 2 Diabetes mellitus. Serum E-selectin could be used as a single putative biomarker of impending nephropathy in insulin resistant type 2 Diabetes mellitus, in the light of albuminuria and e-GFR thereby signaling the arrival of newer perspectives in the management of renal complications related to insulin resistance. Our research corresponds to a basic attempt to comprehend as to how IR and nitric oxide relate to diabetic nephropathy, with reference to proteinuria. Nitric oxide (NO) levels were perceptibly pronounced in T2DM. The most important finding is that NO correlates positively with Urine Albumin-to- Creatinine ratio and HOMA-IR, the surrogate marker of insulin resistance. In the current scenario, a few therapeutic drugs for Diabetic nephropathy are available. However, the armamentarium largely comprises conventional anti-hypertensives, besides antidiabetic formulations. Having said that, itremains to be seen as to whether such modalities would confer optimal benefits. There exists a possibility of introducing drugs that could envisage benefits beyond the horizons of glycemic control, IR and blood pressure.

11. NOVELTY OF THE STUDY

1. Till date, very few reports are available citing the predictive capacity of a sensitive and specific biomarker namely Nitrotyrosine (NT) to denote impending vascular complications in diabetic nephropathy. Moreover, our study has correlated NT levels with albumin creatinine ratio, an objective marker of nephropathy. We have further demonstrated that no additional parameters of oxidative stress are required other than NT to confirm the utility of a sensitive and specific biomarker of oxidative stress that would reveal impending vascular complications linked to diabetic nephropathy. This fact acquires relevance in pharmacogenomics and personalized medicine.
2. E-selectin levels could prove to be a putative and novel biomarker of nephropathy in type 2 diabetic patients, especially at a juncture when very few simple, economically viable, specific, and sensitive predictors of IR induced diabetic nephropathy are available in the existing armamentarium.
3. The two cardinal markers of chronic kidney disease are Urine Albumin-to-Creatinine ratio and estimated glomerular filtration rate (eGFR). Though studies performed by earlier workers point to the assessment of urine albumin excretion annually so as to monitor renal damage in patients with T2DM, ambiguity still exists in its clinical applications. Recent evidences suggest that combined changes in albuminuria and eGFR over prolonged duration of diabetes are strongly associated with future risk of kidney failure in patients with type 2 diabetes.
4. This study has endeavored to integrate the markers of endothelial dysfunction, namely ET-1 and VEGF-A in the light of IR and insulin sensitivity, but as a function of UACR and e-GFR. This we feel might signal the advent of a novel and concerted approach that would enable us to monitor the progression intensely and closely to end-stage kidney disease (ESKD)
5. The present study acquires special significance for justifying the inclusion of nitric oxide as a single, reliable marker of oxidative stress mediated endothelial dysfunction in diabetic nephropathy, in the light of the various facts includes, With reference to the usual measurement of Malondialdehyde (MDA) as a product of oxidative stress mediated lipid peroxidation, the non-

specificity of Thiobarbituric acid Reactive Substances(TBARS) assay reactivity on MDA and production of MDA from reactions other than lipid peroxidation continues to be a major hampering factor.Furthermore, the low stability of MDA is attributed to its enhanced tendency for reacting with other biomolecules besides rapid enzymatic degradation.. Present study has convincingly demonstrated that Nitric oxide can be considered as a single reliable marker of endothelial dysfunction and could replace MDA in monitoring oxidative stress related endothelial dysfunction in insulin resistant T2DM.

12. LIMITATIONS OF THE STUDY

1. The products of oxidative stress and inflammatory indicators represent the primary causes of endothelial dysfunction in diabetes mellitus, but we did not measure inflammatory indexes. Further research is needed to determine the relationship between the specific risk variables and severity of diabetic nephropathy.
2. Sample size taken for the control and test groups was low.
3. The study did not include other conventional markers of oxidative stress in type 2 Diabetes mellitus.

13. FUTURE SCOPE

1. NT & E-selectin levels could be used diagnostically as a novel/putative biochemical predictor of nephropathy in type 2 diabetic patients,
2. The present study has provided an insight into the suggested role of drugs that could modulate NO production and action with reference to the management of diabetic nephropathy(DN). This would usher in a fresh approach to treat DN, besides expanding the horizons in the pharma sector and clinical pharmacology. In addition, promulgation of the relatively safe use of NO in therapeutics might open newer vistas in confronting the perils of DN.
3. The present study could acquire relevance in pharmacology and pharma industry, where therapeutic formulations could be preparedand targeted at VEGF –A and ET-1 as a whole, in addition to the standard care that is promulgated for not only controlling DM but preventing the catastrophe of diabetic nephropathy .Our study has endeavoured to integrate the markers of endothelial dysfunction, namely ET-1 and VEGF-A in the light of insulin resistance and insulin sensitivity but as a function of UACR and eGFR. This we feel might signal the advent of a novel and concerted approach that would enable us to intensely and closely monitor the progression to end-stage kidney disease(ESKD)

14. REFERENCES

1. Sami W, Ansari T, Butt NS, Hamid MRA. Effect of diet on type 2 diabetes mellitus: Areview. Int J Health Sci (Qassim). 2017 Apr-Jun;11(2):65-71. PMID: 28539866; PMCID: PMC5426415.
2. American Diabetes association .Diabetes Care .2013 ;36 (1): S67-S74
3. International Diabetes Federation IDF Diabetes Atlas. Epidemiology and Mobidity. In: International Diabetes Federation. Available from http://www.idf.org/. Accessed on 1March 2011 [Google Scholar]
4. Hu FB. Globalization of diabetes: the role of diet, lifestyle, and genes. Diabetes Care. 2011 Jun;34(6):1249-57. doi: 10.2337/dc11-0442. PMID: 21617109; PMCID: PMC3114340.
5. Roy CK, Ojha JK, Bajpai HS. A review of the history of prameha and diabetes mellitus. AncSci Life. 1993 Jan;12(3-4):394-8. PMID: 22556618; PMCID: PMC3336561.
6. Rao AV. Mind in ayurveda. Indian J Psychiatry. 2002 Jul;44(3):201-11. PMID:21206574; PMCID: PMC2954390.
7. Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK. Significance ofHbA1c Test in Diagnosis and Prognosis of Diabetic Patients. Biomark Insights. 2016Jul 3;11:95-104. doi: 10.4137/BMI.S38440. PMID: 27398023; PMCID: PMC4933534.
8. Quianzon CC, Cheikh I. History of insulin. J Community Hosp Intern Med Perspect. 2012 Jul 16;2(2). doi: 10.3402/jchimp.v2i2.18701. PMID: 23882369; PMCID: PMC3714061.
9. Steiner, D.F. 1967. Evidence for a precursor in the biosynthesis of insulin. Trans. N. Y. Acad. Sci. 30:60-68.
10. Luo X, Jiang L, Du B, Wen Y, Wang M, Xi X., Beijing Acute Kidney Injury Trial(BAKIT) workgroup. A comparison of different diagnostic criteria of acute kidney injury in critically ill patients. Crit Care. 2014 Jul 08;18(4):R144. [PMC free article][PubMed]
11. Sefah IA, Okotah A, Afriyie DK, Amponsah SK. Adherence to Oral Hypoglycemic Drugs among Type

12. KaurJ. 2014. Cardiology Research and Practice, Volume 2014, ArticleID-943162,21-pages. http:// dx.doi. org/ 10. 1155 /2014 /943162.
13. Diabetic Patients in a Resource-Poor Setting. Int J Appl Basic Med Res. 2020 Apr-Jun;10(2):102-109. doi: 10.4103/ijabmr.IJABMR_270_19. Epub 2020 Apr 2. PMID: 32566526; PMCID:PMC7289204.
14. Ghazanfari Z, Haghdoost AA, Alizadeh SM, Atapour J, Zolala F. A Comparison of HbA1c and Fasting Blood Sugar Tests in General Population. Int J Prev Med. 2010 Summer;1(3):187-94. PMID: 21566790; PMCID: PMC3075530
15. Babu,S,Krishnan,M,Rajagopal,P,Periyasamy,V,Veeraraghavan,V,Govindan,R, &Jayaraman etal-Sitosterol attenuates insulin resistance in adipose tissue via IRS 1/Akt mediated insulin signalingin high fat diet and sucrose induced type2 diabetic rats'European journal ofpharmacology,873,173004.
16. Eboh, C., Tahseen, A. Chowdhury. Management of diabetic renal disease. Ann Transl Med. 2015; 3(11): 154:1-8
17. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010 Oct 29;107(9):1058-70. doi: 10.1161/CIRCRESAHA.110.223545. PMID: 21030723; PMCID: PMC2996922.
18. Tufro A, Veron D. VEGF and podocytes in diabetic nephropathy. SeminNephrol. 2012 Jul;32:385-93. doi: 10.1016/j.semnephrol.2012.06.010. PMID: 22958493; PMCID: PMC3438453.
19. García-Carro C, Vergara A, Bermejo S, Azancot MA, Sánchez-Fructuoso AI, Sánchez dela Nieta MD, Agraz I, Soler MJ. How to Assess Diabetic Kidney Disease Progression? From Albuminuria to GFR. J Clin Med. 2021 Jun 5;10(11):2505. doi: 10.3390/jcm10112505. PMID: 34198818; PMCID: PMC8201333
20. Mijatović S, Savić-Radojević A, Plješa-Ercegovac M, Simić T, Nicoletti F, Maksimović-Ivanić D. The Double-Faced Role of Nitric Oxide and Reactive Oxygen Species in Solid Tumors. Antioxidants. 2020; 9:374.
21. Kaur, J 2014 'A Comprehensive Review on Metabolic Syndrome' Cardiology Research and Practice,1–21.
22. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2014 Jan;37Suppl 1:S81-90. doi: 10.2337/dc14-S081. PMID: 24357215.

23. Harding JL, Pavkov ME, Magliano DJ, Shaw JE, Gregg EW. Global trends in diabetes complications: A review of current evidence. Diabetologia (2019) 62(1):3–16. doi: 10.1007/s00125-018-4711-2)
24. Marshall CB. Rethinking glomerular basement membrane thickening in diabetic nephropathy: adaptive or pathogenic? Am J Physiol Renal Physiol. 2016 Nov 1;311(5):F831-F843. doi:10.1152/ajprenal.00313.2016. Epub 2016 Aug 31. PMID: 27582102; PMCID: PMC6121820.
25. Levey, A.S., de Jong, P.E., Coresh, J., Nahas, M., Astor, B.C. The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. Kidney Int. 2011; 80: 17-28.
26. Ciobanu DM, Bala C, Rusu A, Cismaru G, Roman G. E-Selectin Is Associated with Daytime and 24-Hour Diastolic Blood Pressure Variability in Type 2 Diabetes. Biomedicines. 2022 Jan 26;10(2):279. doi: 10.3390/biomedicines10020279. PMID: 35203490; PMCID: PMC8869192.
27. Abdel-Raheem A, Hamed HI, Fahim E, Mohamed AS. Oxidative stress markers as early predictors of diabetes complications in Type 2 diabetic patients. Indian J PhysiolPharmacol2022;66:111-9.
28. Blanchard-Fillion B, Prou D, Polydoro M, Spielberg D, Tsika E, Wang Z, Hazen SL, Koval M, Przedborski S, Ischiropoulos H. Metabolism of 3-nitrotyrosine induces apoptotic death in dopaminergic cells. J Neurosci. 2006 Jun 7;26(23):6124-30. doi: 10.1523/JNEUROSCI.1038-06.2006.PMID: 16763020; PMCID: PMC6675196.
29. Goswami B, Sarkar S, Sengupta S, Bhattacharjee B. Assessment of serum nitric oxide level and its correlation with anthropometric parameters and lipid profile in diabetic patients: A hospital-basedstudy from Tripura. J Family Med Prim Care. 2022 Sep;11(9):5161-5165. doi:10.4103/jfmpc.jfmpc_2395_21. Epub 2022 Oct 14. PMID: 36505578; PMCID: PMC9731029.
30. Gan LM, Lagerström-Fermér M, Carlsson LG, Arfvidsson C, Egnell AC, Rudvik A, Kjaer M, Collén A, Thompson JD, Joyal J, Chialda L, Koernicke T, Fuhr R, Chien KR, Fritsche-Danielson R. Intradermal delivery of modified mRNA encoding VEGF-A in patients with type 2 diabetes. Nat Commun. 2019Feb 20;10(1):871. doi: 10.1038/s41467-019-08852-4. PMID: 30787295; PMCID: PMC6382754.

31. Wu SF, NorenHooten N, Freeman DW, Mode NA, Zonderman AB, Evans MK. Extracellular vesicles in diabetes mellitus induce alterations in endothelial cell morphology and migration. J Transl Med. 2020 : Jun 9;18(1):230. doi: 10.1186/s12967-020-02398-6. PMID: 32517700; PMCID: PMC7285586.
32. Charan J, Biswas T. How to Calculate Sample Size for Different Study Designs inMedical Research?Indian J Psychol Med [Internet]. 35(2). Available from:www.ijpm.info.
33. Keys A, Fidanza F, Karvonen MJ, Kimura N, Taylor HL. Indices of relative weight and obesity. JChronDis. 1972; 25(6): 329– 343.
34. World Health Organisation. Physical status: the use and interpretation of anthropometry: report of a WHO Expert Committee (WHO, 1995).
35. World Health Organization. Waist Circumference and Waist- Hip Ration:Report of a WHO Expert Consultation. WorldHealthOrganization;Geneva,Switzerland:2008
36. StamlerJ.Bloodpressureandhighbloodpressure.Aspectsof risk. *Hypertension.* 1991;18(3 Suppl):I95.doi: 10.1161/01.HYP.18.3_Suppl.I95.
37. Ambade VN, Sharma YV, Somani BL. methods for estimation of blood glucose: a comparative evaluation. Med J Armed Forces India. 1998 Apr;54(2):131-133. doi: 10.1016/S0377-1237(17)30502-6. Epub 2017 Jun 26. PMID: 28775446; PMCID: PMC5531325.
38. Zawada RJ, Kwan P, Olszewski KL, Llinas M, Huang SG. Quantitative determination of urea concentrations in cell culture medium. Biochem Cell Biol. 2009 Jun;87(3):541-4. doi: 10.1139/o09-011. PMID: 19448747; PMCID: PMC2804236.
39. Syal K, Banerjee D, Srinivasan A. Creatinine estimation and interference. Indian J ClinBiochem. 2013 Apr;28(2):210-1. doi: 10.1007/s12291-013-0299-y. Epub 2013 Jan 26. PMID: 24426213; PMCID: PMC3613509.
40. Corso G, Papagni F, Gelzo M, Gallo M, Barone R, Graf M, Scarpato N, Dello Russo A. Development and Validation of an Enzymatic Method for Total Cholesterol Analysis Using Whole Blood Spot. J ClinLab Anal. 2016 Sep;30(5):517-23. doi: 10.1002/jcla.21890. Epub 2015 Oct 29. PMID: 26511311; PMCID: PMC6807098.

41. Penumarthy S, Penmetsa GS, Mannem S. Assessment of serum levels of triglycerides, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol in periodontitis patients. J Indian SocPeriodontol. 2013 Jan;17(1):30-5. doi: 10.4103/0972-124X.107471. PMID: 23633769; PMCID: PMC3636940.
42. Herrmann W, Schütz C, Reuter W. ZurBestimmung des HDL-Cholesterols [Determination of HDL-cholesterol]. Z Gesamte Inn Med. 1983 Jan 1;38(1):17-22. German. PMID: 6342279.
43. Krishnaveni P, Gowda VM. Assessing the Validity of Friedewald's Formula and Anandraja's Formula For Serum LDL-Cholesterol Calculation. J ClinDiagn Res. 2015 Dec;9(12):BC01-4. doi: 10.7860/JCDR/2015/16850.6870. Epub 2015 Dec 1. PMID: 26816879; PMCID: PMC4717736.
44. Genc S, Omer B, Aycan-Ustyol E, Ince N, Bal F, Gurdol F. Evaluation of turbidimetric inhibition immunoassay (TINIA) and HPLC methods for glycated haemoglobin determination. J Clin Lab Anal. 2012 Nov;26(6):481-5. doi: 10.1002/jcla.21550. PMID: 23143632; PMCID: PMC6807436.
45. Singh A, Satchell SC. Microalbuminuria: causes and implications. PediatrNephrol. 2011 Nov;26(11):1957-65. doi: 10.1007/s00467-011-1777-1. Epub 2011 Feb 8. PMID: 21301888; PMCID: PMC3178015.
46. Syal K, Banerjee D, Srinivasan A. Creatinine estimation and interference. Indian J ClinBiochem. 2013Apr;28(2):210-1. doi: 10.1007/s12291-013-0299-y. Epub 2013 Jan 26. PMID: 24426213; PMCID: PMC3613509.
47. Erman A, Rahamimov R, Mashraki T, Levy-Drummer RS, Winkler J, David I, Hirsh Y, Gafter U, Chagnac A. The urine albumin-to-creatinine ratio: assessment of its performance in the renal transplant recipient population. Clin J Am SocNephrol. 2011 Apr;6(4):892-7. doi: 10.2215/CJN.05280610. Epub 2011 Jan 6. PMID: 21212424; PMCID: PMC3069384.
48. Høier R, Jensen AL. Evaluation of an enzyme linked immunosorbent assay (ELISA) for Determinationof insulin in dogs. ZentralblVeterinarmed A. 1993 Feb;40(1):26-32. doi: 10.1111/j.1439-0442.1993.tb00597.x. PMID: 8451901.
49. Knight AR, Taylor EL, Lukaszewski R, Jensen KT, Jones HE, Carré JE, Isupov MN, Littlechild JA, Bailey SJ, Brewer E, McDonald TJ, Pitt AR, Spickett CM,Winyard PG. A high-sensitivity electrochemiluminescence-based

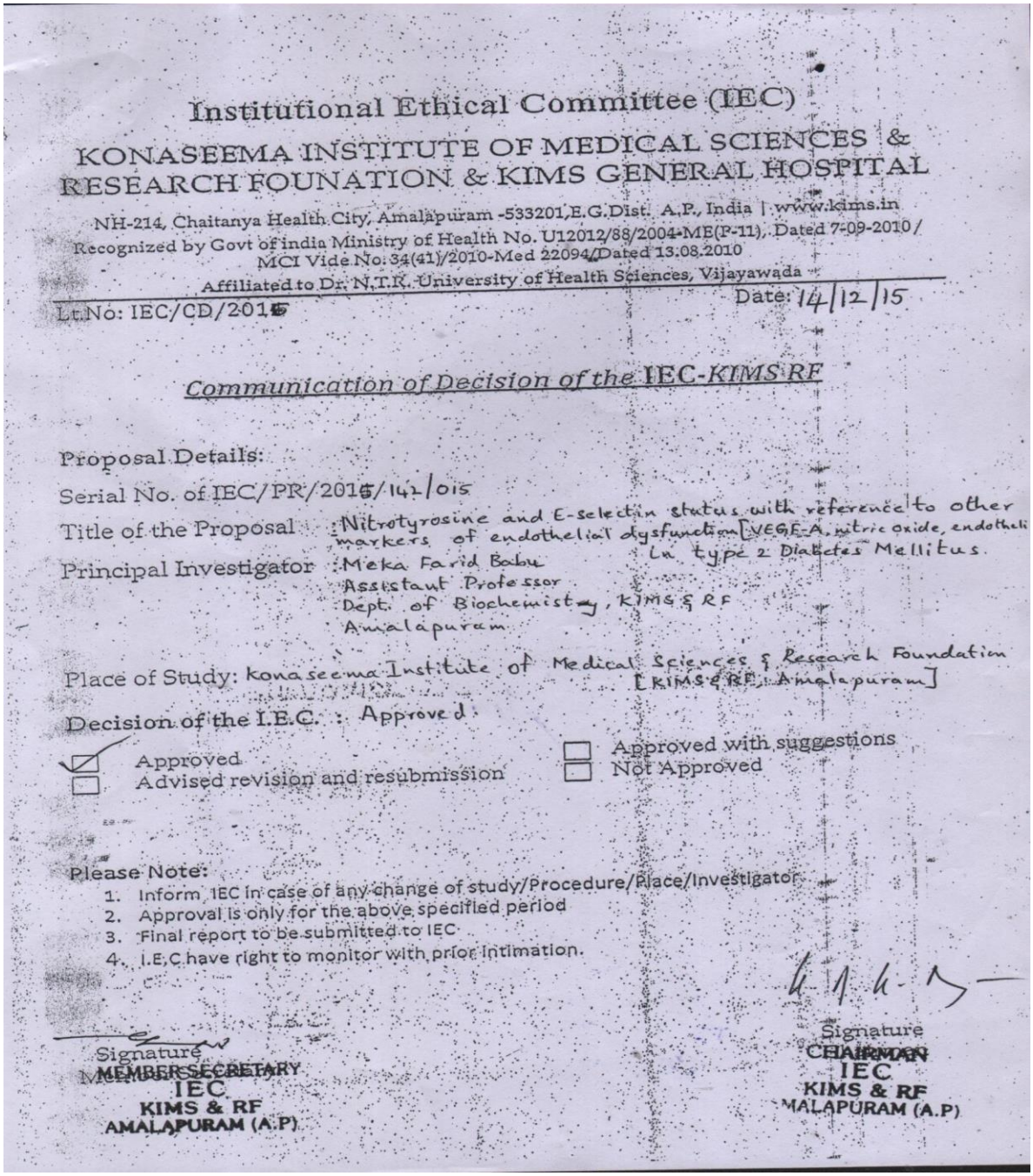
ELISA for the measurement of the oxidative stress biomarker, 3-nitrotyrosine, in human blood serum and cells. Free RadicBiol Med. 2018 May 20;120:246-254. doi: 10.1016/j.freeradbiomed.2018.03.026. Epub 2018 Mar 17. PMID: 29555590.

50. Lundberg AH, Granger DN, Russell J, Sabek O, Henry J, Gaber L, Kotb M, Gaber AO. Quantitative measurement of P- and E-selectin adhesion molecules in acute pancreatitis: correlation with distant organ injury. Ann Surg. 2000 Feb;231(2):213-22. doi: 10.1097/00000658-200002000-00010. PMID: 10674613; PMCID: PMC1420989.
51. Nowacka A, Smuczyński W, Rość D, Woźniak-Dąbrowska K, Śniegocki M. Serum VEGF-A concentrations in patients with central nervous system (CNS) tumors. PLoS One. 2018 Mar 28;13(3):e0192395. doi: 10.1371/journal.pone.0192395. PMID: 29590109; PMCID: PMC5873928.
52. Khalid W, Varghese SS, Sankari M, Jayakumar ND. Comparison of Serum Levels of Endothelin-1 in Chronic Periodontitis Patients Before and After Treatment. J ClinDiagn Res. 2017 Apr;11(4):ZC78- ZC81. doi: 10.7860/JCDR/2017/24518.9698. Epub 2017 Apr 1. PMID: 28571268; PMCID: PMC5449924.
53. Bryan NS, Grisham MB. Methods to detect nitric oxide and its metabolites in biological samples. Free RadicBiol Med. 2007 Sep 1;43(5):645-57. doi: 10.1016/j.freeradbiomed.2007.04.026. Epub2007 Apr 29. PMID: 17664129; PMCID: PMC2041919.
54. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man.*Diabetologia.*1985;28:4129.
55. Wallace,T.M., Levy,J.C., Matthews ,D.R., Use and abuse of HOMA modeling. Diabetes Care. 2004 Jun;27(6):1487-95. doi: 10.2337/diacare.27.6.1487. PMID: 15161807.
56. Hrebícek, J., Janout, V., Malincíková, J., Horáková ,D., Cízek, L., Detection of insulin resistance by simple quantitative insulin sensitivity check index QUICKI for epidemiological assessment and prevention ,2002 Jan;87(1):144-7. doi: 10.1210/jcem.87.1.8292. PMID: 11788638.

57. Das, S.K., Roy, D.K., Chowdhury,A.A., Roy, A.S., Ahammed ,S.U., Asadujjaman,M., Rabbani,M.G., Islam ,M.S., Barman,G.C., Chanda, K., Hossain, M.B., Salahuddin,A.Z., Saha, S., Das ,S.K., Saha ,S., Ara,J., Correlation of eGFR By MDRD and CKD-EPI Formula with Creatinine Clearance Estimation in CKD Patients and Healthy Subjects. Mymensingh Med J. 2021 Jan;30(1):35-42. PMID: 33397848.
58. Dobrian AD, Davies MJ, Schriver SD, Lauterio TJ, Prewitt RL. Oxidative stress in a rat model of obesity-induced hypertension. Hypertension. 2001;37:554–560.
59. Pacher P, Obrosova I, Mabley J, Szabó C. Role of nitrosative stress and peroxynitrite in the pathogenesis of diabetic complications: emerging new therapeutical strategies. Current Medicinal Chemistry. 2005;12(3):267–275.
60. Xue P., Hou Y., Chen Y., Yang B., Fu J., Zheng H., Yarborough K., Woods C.G., Liu D., Yamamoto M., et al. Adipose deficiency of Nrf2 in ob/ob mice results in severe metabolic syndrome. Diabetes.2012;62:845–854m. Nat Rev Endocrinol 2012;8 (4): 237-245.
61. Laakso,M., Kuusisto, J., Insulin resistance and hyperglycaemia in cardiovascular disease development. Nat Rev Endocrinol. 2014 May;10(5):293-302. doi: 10.1038/nrendo.2014.29. Epub2014 Mar 25. PMID: 24663222.
62. Jude, E.B., Douglas,J.T., Anderson, S.G., Young, M.J., Boulton ,A.J., Circulating cellular adhesion molecules ICAM-1, VCAM-1, P- and E-selectin in the prediction of cardiovascular disease in diabetes mellitus. Eur J Intern Med. 2002 May;13(3):185-189. doi: 10.1016/s0953-6205(02)00014-6. PMID:12020626.
63. Yang J, Liu Z. Mechanistic Pathogenesis of Endothelial Dysfunction in Diabetic Nephropathy and Retinopathy. Front Endocrinol (Lausanne). 2022 May 25;13:816400. doi: 10.3389/fendo.2022.816400. PMID: 35692405; PMCID: PMC9174994.
64. Cole JB, Florez JC. Genetics of diabetes mellitus and diabetes complications. Nat Rev Nephrol. 2020Jul;16:377-390. doi: 10.1038/s41581-020-0278-5. Epub 2020 May 12. PMID: 32398868; PMCID: PMC9639302.

65. Tufro A, Veron D. VEGF and podocytes in diabetic nephropathy. SeminNephrol. 2012 Jul;32:385-93. doi: 10.1016/j.semnephrol.2012.06.010. PMID: 22958493; PMCID: PMC3438453.
66. Serena Salvatore and Enzo Maria Vingolo. J Ophthalmol. 2010: 354645. Published online 2011 Mar 3. doi: 10.1155/2010/354645. PMCID:PMC3065050
67. Ketonen J, Shi J, Martonen E, Mervaala E. Periadventitial adipose tissue promotes endothelial dysfunction via oxidative stress in diet-induced obese C57Bl/6 mice. Circ J. 2010;74:1479–1487.

15. APPENDIX



Institutional Ethical Committee (IEC)

KONASEEMA INSTITUTE OF MEDICAL SCIENCES & RESEARCH FOUNATION & KIMS GENERAL HOSPITAL

NH-214, Chaitanya Health City, Amalapuram -533201,E.G.Dist. A.P., India | www.kims.in
Recognized by Govt of india Ministry of Health No. U12012/88/2004-ME(P-11), Dated 7-09-2010 / MCI Vide No: 34(41)/2010-Med 22094/Dated 13.08.2010
Affiliated to Dr. N.T.R. University of Health Sciences, Vijayawada

Lt.No: IEC/CD/2015 Date: 14/12/15

Communication of Decision of the IEC-KIMS RF

Proposal Details:

Serial No. of IEC/PR/2015/142/015

Title of the Proposal : Nitrotyrosine and E-selectin status with reference to other markers of endothelial dysfunction [VEGF-A, nitric oxide, endotheli in type 2 Diabetes Mellitus.

Principal Investigator : Meka Farid Babu
Assistant Professor
Dept. of Biochemistry, KIMS & RF
Amalapuram

Place of Study: konaseema Institute of Medical Sciences & Research Foundation [KIMS&RF, Amalapuram]

Decision of the I.E.C. : Approved

☑ Approved
☐ Advised revision and resubmission
☐ Approved with suggestions
☐ Not Approved

Please Note:
1. Inform IEC in case of any change of study/Procedure/Place/Investigator
2. Approval is only for the above specified period
3. Final report to be submitted to IEC
4. I.E.C have right to monitor with prior intimation.

Signature
MEMBER SECRETARY
IEC
KIMS & RF
AMALAPURAM (A.P)

Signature
CHAIRMAN
IEC
KIMS & RF
MALAPURAM (A.P)

SBV/Ph.D. Methodology/2017/2025 08.02.2017

To

Mr.Meka Farid Babu (UIN No: 1505002004)
Ph.D. Student,
Biochemistry,
SBV.

Sir,

Sub: Grant of exemption to Mr.Meka Farid Babu (Doctoral Student) from Ph.D. Part I Methodology examinations.

With reference to the above subject and based on the application of the candidate duly endorsed by the Guide, Mr.Meka Farid Babu is exempted from appearing at the University Research Methodology Examination (Paper I and Paper II) to be held in March 2017.

The above exemption is issued in accordance with PhD. Regulations of SBV.

8/2/17

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Certificate of Participation

This is to certify that Dr/Mr/Mrs. Meka Farid Babu, Asst. Professor of BIOCHEMISTRY DEPARTMENT has attended the SMARC Life Science Conference at KIMS & RF, Amalapuram and participated in the event of "POSTER PRESENTATION", entitled: "Study of Nitrotyrosine levels in type 2 diabetic Patients Compared with healthy Individuals."

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has participated at the National workshop on

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in association with Center for Health Professions Education (CHPE)

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This is to certify that Mr./~~Ms./Dr.~~ Fazid Babu Meka, KIMS, A.P has successfully completed the 10 days International online Internship cum Certificate course on **"Techniques in Molecular Diagnostics (TMD)"** jointly organised by Centre for Bioscience and Nanoscience Research (CBNR) and Karpagam Academy of Higher Education (Deemed to be University), Eachanari, Coimbatore, Tamil Nadu, India, from 08/10/2021 to 23/10/2021.

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AMALAPURAM
ANDHRA PRADESH - 533201
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Director-General, Indian Council of Medical Research
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Research article

3-Nitrotyrosine (NT) levels in serum and its association with insulin resistance in patients with type 2 diabetes mellitus: Biomarker role of NT in the assessment of oxidative stress mediated impending vascular complications in nephropathy

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ABSTRACT

Introduction and Aim: 3-Nitrotyrosine (NT) has been recognized as a marker of oxidative stress in diabetes mellitus. NT has also been studied in diverse metabolic conditions. The aim of our study was oriented towards the role of NT as a predictor of oxidative stress mediated impending nephropathy in diabetes mellitus and that with reference to albuminuria.

Materials and Methods: A total of 150 type 2 diabetics in the age group 35 - 50 years were enrolled as three groups, comprising 50 each, based on albuminuria. 50 healthy age and gender matched subjects constituted the control group. Serum NT and Insulin were assessed by ELISA. HbA1c was quantitated by immunoturbidimetric method and microalbumin was assessed by turbilatex method. Routine biochemistry was enabled through ERBA EM-200 fully automated analyzer. Stringent quality control was affected. The study was begun following approval accorded by the competent committees.

Results: NT levels were positively correlated with albumin-creatinine ratio and insulin resistance. NT could be used as a predictor of impending vascular complications in diabetic nephropathy.

Conclusion: NT levels could act as a predictor of oxidative stress mediated diabetic nephropathy in the light of albuminuria.

Keywords:3-Nitrotyrosine (NT); Type 2 diabetes mellitus (T2DM); Glycemic control; Insulin resistance (IR); HOMA-IR; nephropathy.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is an endocrine disorder which is presently experiencing an alarming increase in developing countries. The prevalence of T2DM in developed countries is 1.2%, whereas in developing countries, the prevalence is believed to be fourfold higher (1,2). The aggressive multipronged approach would result in an attenuated risk for microangiopathy, coronary events and mortality. However, the incidence of end stage renal disease (ESRD) is progressively on the rise globally. To evaluate the progression of diabetic nephropathy, monitoring of albuminuria and glomerular filtration rate (GFR) are appropriately indicated by the KDIGO: Kidney Disease Improving Global Outcomes CKD Work Group (3). Repeated monitoring of albuminuria is emphatically recommended in routine clinical practice in diabetes and pronounced decline in GFR in both type 1 and type 2 diabetes has been documented (4). However, it is practically difficult to predict the progression of diabetic nephropathy by quantitating albuminuria and eGFR (5,6). Precisely for this reason, the authors of the present paper had embarked on a study to look for sensitive and specific biochemical marker for assessing impending vascular complications in diabetic nephropathy. Presently, very few reports with the aforesaid focus are available in the literature.

Acute hyperglycemia increases the levels of proinflammatory cytokines including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), (7-9). Nitrotyrosine (NT) is indirectly inferred as a marker of oxidative stress because of the production of peroxynitrite, also called as peroxonitrite ($ONOO^{-}$) during acute hyperglycemia (10). Experimental studies have also revealed that elevated NT is associated with coronary artery disease (CAD) (11-13). It is well documented that oxidative stress and the pathogenesis of insulin resistance through insulin signal inhibition and adipocytokines dysregulation are considered cardinal (14,15). However, very few reports are available from South India pertaining to our present attempt, namely, to look out for a biomarker, with reference to imminent nephropathy in type 2 diabetics. Hence, we decided to explore the NT levels in the serum of patients with T2DM and its association with microalbuminuria and insulin

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Research article

Extracellular E-selectin levels and its association with insulin resistance: Putative role of E-selectin as a biomarker of oxidative stress linked vascular manifestations in diabetic nephropathy

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ABSTRACT

Introduction and aim: E-selectin, a cell adhesion molecule depicting nexus with oxidative stress in hyperglycemia has been documented in several other clinical conditions. The present study centers around the putative role of E-selectin as a biomarker of diabetic nephropathy linked to oxidative stress.

Materials and Methods: 150 type 2 diabetics in the age group of 35 - 50 years were classified into three groups, comprising 50 each, based on albuminuria. Fifty healthy age and gender matched individuals constituted the control. Extracellular E-selectin and Insulin were quantitated appropriately. Measurement of HbA1c and microalbumin were also enabled. Routine biochemistry analyses were undertaken, based on established automated procedures. The study was initiated only following approval accorded by the Institutional Ethics Committee.

Results: E-selectin levels in serum were significantly associated with Urine Albumin-to-Creatinine Ratio (UACR) and Insulin resistance in the light of estimated Glomerular Filtration Rate.

Conclusion: E-selectin levels could thus act as a putative biomarker of diabetic nephropathy linked to oxidative stress, as observed in insulin resistant type 2 diabetes.

Keywords: E-selectin; type 2 diabetes mellitus (T2DM); glycemic control; insulin resistance (IR); HOMA-IR; nephropathy.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a metabolic disorder which has particularly been associated, in recent years, with a pronounced increase in developing countries (1). It must be said that aggressive intervention in patients with type 2 diabetes mellitus would eventually result in an attenuated risk of complications that include among others microangiopathy and cardiovascular diseases.

Evaluation of albuminuria and glomerular filtration rate (GFR) has become the mainstay, as recommended by the KDIGO: Kidney Disease Improving Global Outcomes CKD Work Group. The evaluation is with reference to end stage renal disease (ESRD) which is presently on the rise globally and hence it is imperative to monitor the progression of diabetic nephropathy (2). Careful monitoring of albuminuria is advocated in routine practice in diabetes, and it is underlined that GFR declines in both type 1 and type 2 diabetes (3). In such instances, however, it is not pragmatic to predict the natural course of diabetic nephropathy based on albuminuria and eGFR (4). It is with this perspective that the study was planned with a view to documenting the role of E-selectin as an objective biomarker, in combination with the conventional parameters, namely albuminuria and eGFR. This would fortify the prediction of vascular outcome as observed in diabetic nephropathy, thereby acquiring distinct relevance in clinical endocrinology and evidence-based medicine.

Acute hyperglycemia has a nexus with circulating proinflammatory cytokines, namely tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (5). E-selectin is considered as an indirect marker of oxidative stress that could be attributed to hyperglycemia (6). Evidence points to the fact that elevated E-selectin is associated with coronary artery disease (CAD) (7). Oxidative stress and insulin resistance mediate pathways related to the inhibition of insulin signal and dysregulation of adipocytokines. These points acquire cardinal relevance (8). It must be stated in all fairness that very few reports are available from South India pertaining to our present attempt, namely with reference to E-selectin as a biochemical marker of imminent nephropathy in type 2 diabetes. Hence, we decided to track the extracellular E-selectin levels and its association with microalbuminuria, insulin resistance and eGFR with a view towards the identification of an objective and reliable biomarker for impending diabetic nephropathy.

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ASSOCIATION BETWEEN INSULIN RESISTANCE AND ENDOTHELIAL DAMAGE: A BIOCHEMICAL STUDYWITH REFERENCE TO THE VARIOUSSTAGES OF DIABETIC NEPHROPATHY

MekaFarid Babu[1], Srinivasan AR[2*],Benerji GV[3]

Article History: Received: 02.07.2023 Revised: 15.07.2023 Accepted: 23.07.2023

Abstract

Introduction and Aim: Endothelin-1 (ET-1) and Vascular Endothelial Growth Factor-A (VEGF-A) have been recognized as markers of endothelial dysfunction in diabetes mellitus. VEGF-A and ET-1 are vasoactive peptides that have a nexus with vascular manifestations including optic neuropathies and disturbances in retinal circulation. This study compared vascular dysregulatedmetrics,VEGF-A and ET-1 with insulin resistance and sensitivity, in the light of riskfactors for diabetic nephropathy in a graded manner, based on albuminuria.

Subjects and Methods: A total of 90 type 2 diabetics in the age group 35 -50 years were enrolled as three groups, comprising 30 each, based on albuminuria. 30 healthy age and gender matched subjects constituted the control group.VEGF-A, ET-1 and Insulin were quantitated by ELISA. Microalbumin was estimated by turbilatex method. Routine biochemistry analyses wascarried out on fully automated analyzer. Stringent quality control was promulgated . The study was begun following approval accorded by the competent authority.

Results: The dysregulated metricspositivelycorrelated with HOMA-IR (Insulin resistance)andalbumin-creatinine ratio.; VEGF-A, ET-1 negatively correlatedwith HOMA-β (Beta cell mass), QUICKI (Insulin sensitivity)and eGFR.Thus, VEGF-A and ET-1 might find clinical utility as indicators of imminent vascular problems.

Conclusion:VEGF-A and ET-1, with reference to theindicators of insulin resistance, insulin sensitivity and Beta cell mass would acquire significance, in the light of albuminuria, as biomarkers in angiopathy related to diabetic nephropathy

Key words:HOMA-IR, HOMA –β, QUICKI, eGFR, VEGF-A,endothelin-1,diabetic nephropathy.

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NITRIC OXIDE AS AN OBJECTIVE MARKER OF OXIDATIVE STRESS MEDIATED ENDOTHELIAL DYSFUNCTION IN NEPHROPATHY: A STUDY ON TYPE 2 DIABETES MELLITUS WITH REFERENCE TO INSULIN RESISTANCE, URINARY ALBUMIN CREATININE RATIO AND E GFR

Meka Farid Babu[1], Srinivasan AR[2*],Benerji GV[3]

Article History: Received: 02.07.2023 Revised: 15.07.2023 Accepted: 23.07.2023

Abstract

Background: Thenexus among urine protein and serum nitric oxide, insulin resistance has beenevaluated in type 2 diabetics by earlier workers. However, we wanted to elicit the value of nitric oxide as a solitary, reliable marker of oxidative stress mediated endothelial dysfunction in nephropathy.

Methods: Thestudy subjects (n=150 total; both genders) were those who had visited the clinics of a tertiary health care set up in South India.The groups were segregated: fifty patients with normoalbuminuria (<30 mg/g creatinine), and an equal number of patients each with microalbuminuria (30–299 mg/g creatinine), macroalbuminuria (≥300 mg/g creatinine). Fifty healthy age, gender matched subjects constituted the control group.The duration of T2DM in the experimental group was greater than five years.

Results: In comparison to the control group, the magnitude of glycated haemoglobin (HbA1c), insulin resistance and nitric oxide ($p<0.05$) were statistically significant in the study groups, as a function of albuminuria.

Conclusion: It was concluded based onthe correlation analysis of Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), glycated hemoglobin (HbA1c), fasting plasma glucose (FPG) and Postprandial blood glucose (PPBS) with nephropathy markers, namely urinary albumin creatinine ratio (UACR) and eGFR that nitric oxide could serve as a solitary, reliable and objective marker of endothelial dysfunction in oxidative stress related nephropathy. Since insulin resistance and serum nitric oxide are found to be closely related to diabetic nephropathy, they could serve as reliable reference indices for evaluating diabetic nephropathy.

Key words:HOMA-IR, HOMA –β, QUICKI, eGFR, diabetic nephropathy.

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